This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

QH
201
.A52
v.37

B 1,072,835

ORGANIZED 1878 INCORPORATED 1891

APRIL
1918

VOLUME XXXVII NO. 2

TRANSACTIONS

OF THE

AMERICAN MICROSCOPICAL SOCIETY

PUBLISHED QUARTERLY BY THE
AMERICAN MICROSCOPICAL SOCIETY
MENASHA, WISCONSIN
ANNUAL SUBSCRIPTION $3.00 SINGLE COPY $1.00
DOUBLE NUMBERS $2.00

Digitized by Google

Attention of members of the

AMERICAN MICROSCOPICAL SOCIETY

is called to the announcement of Grants
from the Spencer-Tolles Research Fund.
(See TRANSACTIONS, July, 1913)
These awards are to stimulate and aid research in
Microscopic work

1. The Grantee must be a member of the Society.
2. Resulting discoveries must first be offered for publication in the Society Transactions.
3. Application should be made to Dr. Henry B. Ward, Chm., Urbana, Ill.

NOTICE TO MEMBERS

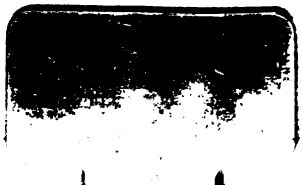
It is a source of regret to the Editor that the *Transactions* cannot be issued as dated. The issue is dependent, however, upon the income of material from the members, and must await upon a certain degree of balance in the material as well as quantity of material.

The Secretary will consider it a favor if members will notify him of non-receipt of numbers of the Transactions, and of changes of address.

Correspondence relative to membership, publication, exchanges, advertising, etc., should be addressed to the Secretary-Editor.

T. W. GALLOWAY,
Secretary-Editor.

Beloit, Wisconsin.



Digitized by Google

TRANSACTIONS

OF THE

American Microscopical Society

ORGANIZED 1878 INCORPORATED 1891

PUBLISHED QUARTERLY

BY THE SOCIETY

EDITED BY THE SECRETARY

T. W. GALLOWAY

BELOIT, WISCONSIN

VOLUME XXXVII

NUMBER TWO

Entered as Second-class Matter December 12, 1910, at the Post-office at Menasha, Wisconsin, under act of March 3, 1879.

The Collegiate Press
GEORGE BANTA PUBLISHING COMPANY
MENASHA, WISCONSIN

Digitized by Google

Digitized by Google

OFFICERS

President: L. E. GRIFFIN.....Pittsburg, Pa.
First Vice President: H. M. WHELPLEY.....St. Louis, Mo.
Second Vice President: C. O. ESTERLY.....Los Angeles, Cal.
Secretary: T. W. GALLOWAY.....Beloit, Wis.
Treasurer: H. J. VAN CLEAVE.....Urbana, Ill.
Custodian: MAGNUS PFLAUM.....Meadville, Pa.

ELECTIVE MEMBERS OF THE EXECUTIVE COMMITTEE

M. M. ELLIS.....Boulder, Colo.
J. E. ACKERT.....Manhattan, Kas.

EX-OFFICIO MEMBERS OF THE EXECUTIVE COMMITTEE

Past Presidents Still Retaining Membership in the Society

ALBERT MCCALLA, PhD., F.R.M.S., of Chicago, Ill., at Chicago, Ill., 1883

GEO. E. FELL, M.D., F.R.M.S., of Buffalo, N. Y., at Detroit, Mich., 1890

SIMON HENRY GAGE, B.S., of Ithaca, N.Y., at Ithaca, N. Y., 1895 and 1906

A. CLIFFORD MERCER, M.D., F.R.M.S., of Syracuse, N. Y., at Pittsburg, Pa., 1896

A. M. BLEILE, M.D., of Columbus, Ohio, at New York City, 1900

C. H. EIGENMANN, Ph.D., of Bloomington, Ind., at Denver, Colo., 1901

E. A. BIRGE, LL.D., of Madison, Wis., at Winona Lake, Ind., 1903

HNERY B. WARD, A.M., Ph.D., of Urbana, Ill., at Sandusky, Ohio, 1905

HERBERT OSBORN, M.S., of Columbus, Ohio, at Minneapolis, Minn., 1910

A. E. HERTZLER, M.D., of Kansas City, Mo., at Washington, D. C., 1911

F. D. HEALD, Ph.D., of Pullman, Wash. at Cleveland, Ohio, 1912

CHARLES BROOKOVER, Ph.D., of Louisville, Ky., at Philadelphia, Pa., 1914

CHARLES A. KOFOID, Ph.D., of Berkeley, Calif., at Columbus, Ohio, 1915

M. F. GUYER, Ph.D., of Madison, Wis., at Pittsburg, Pa., 1917

The Society does not hold itself responsible for the opinions expressed by members in its published *Transactions* unless endorsed by special vote.

Digitized by Google

TABLE OF CONTENTS

FOR VOLUME XXXVII, NUMBER 2, APRIL 1918

Three New Species of Amebas, with Plates, by Asa A. Schaeffer 79

Spermatogenesis of the Dog, with Plate, by Julian Y. Malone 97

Thigmotactic Reactions of the Fresh Water Turbellarian, *Phagocata Gracilis*, Leidy, by Bernol R. Weimer 111

Additions to Our Knowledge of *Unionicola Aculeata* (Koenike), with Plate, by E. C. Faust 125

Notes and Reviews: Methods for Studying Living Trematodes, William Walter Cort; A Substitute for Euparal, E. S. Shepherd; Chromosomes of Ranatra Sp?, A. M. Chickering; Notes on Collecting and Mounting Rotifers; Methods of Preserving Certain Marine Biological Specimens; The Silverman Illuminator for Microscopes 129

Digitized by Google

TRANSACTIONS
OF
American Microscopical Society
(Published in Quarterly Instalments)

Vol. XXXVII APRIL, 1918 No. 2

THREE NEW SPECIES OF AMEBAS: AMOEBA BIGEMMA NOV. SPEC., PELOMYXA LENTISSIMA NOV. SPEC. AND P. SCHIEDTI NOV. SPEC.

ASA. A. SCHAEFFER

The classification of the amebas[1] is peculiar in that two methods of species determination are followed. The larger amebas are classified according to the characteristics possessed by the cytoplasm and the general character of the vegetative stage, while the smaller amebas are being described according to the method of nuclear division prevailing during reproduction. In the larger amebas a study of nuclear division is extremely difficult and for many species impossible, owing for one thing to our ignorance of culture methods by means of which these species could be raised in abundance. On the other hand the small amebas exhibit so little cytoplasmic differentiation that specific determinations on this basis seem impossible.

A specific determination is interesting, however, from at least two points of view. One is the viewpoint of establishing blood relationships of descent between the different species, or systematics proper. The other point of view is a purely practical one, i.e., quick identification. The physiologist or the experimentalist wants a quick and correct method for identifying the organism he is working with. It is obvious therefore that if an ameba possesses characteristic cytoplasmic differentiations which may be observed at any time, the ameba will come to be recognized by these characteristics rather than by a complicated series of nuclear events which occur only occasionally and are frequently made out only with difficulty. In short, as a means of identification, cytoplasmic char-

[1] The word ameba is used as a common name for naked rhizopods lacking internal skeletons such as *Amoeba*, *Pelomyxa*, *Protamoeba*, *Endamoeba*, *Nägleria*, *Hyalodiscus*, *Dinamoeba*, *Vahlkampfia*, etc.

Digitized by Google

acters are preferable where they exist; where these do not exist, recourse may be had to the nuclear division process.

All the larger amebas possess cytoplasmic differentiations in sufficient number and conspicuousness to serve as a ready means of recognition. Many of these characters are subject to very slight variation as a result of changes in the environment. Individual isolation pedigrees carried on for upwards of a hundred linear generations together with many collateral lines under varying food conditions, showed that most of the cytoplasmic characters are hereditary and practically uninfluenced by what might be said to be the most common environmental changes (Schaeffer, Science, 1916, p. 468). Amebas are therefore in this respect like all other groups of animals and the method of classifying them according to cytoplasmic differentiations is therefore sound.

These considerations should convince especially our younger microscopists that the investigation of our larger amebas is not nearly as difficult or forbidding a field as might be imagined from the great amount of labor that has been expended on the study of the life histories and nuclear phenomena of some soil and parasitic amebas during the past decade. The fifty or so species of aquatic amebas thus far described represent beyond any question only a very small fraction of the number of species in existence, and this number of known species could probably be doubled within a few years by careful examination of our marshes and ponds.

AMOEBA BIGEMMA NOV. SPEC.

Diagnosis. Size in locomotion, 100 to 300 microns long. Form very changeable. Pseudopods, numerous, tapering, blunt, never with sharp points. Surface smooth, no fine folds or ridges. Endoplasm usually containing numerous small twin crystals; crystals attached to 'excretion spheres.' Movement rapid, about 125 microns per minute. Nucleus single, spherical or slightly ovoid, about 12 microns in diameter; chromatin in small masses clumped loosely together in the center of the nucleus in a nearly spherical mass about 6.5 microns in diameter. Contractile vacuoles small about 15 microns in diameter; numerous; no coalescence among them; systole slow. Endoplasm filled with small vacuoles. Food: flagellates, ciliates, diatoms, rhizopods, nematodes, vegetal tissue, etc.

This ameba, for which the specific name *bigemma* is proposed, resembles to some extent the figures and descriptions of Parona's *digitata*, Mereschkowsky's *angulata*, Gruber's *spumosa* and Penard's *vespertilio*. In fact I regarded it at first as the *angulata* of Mereschkowsky or the *vespertilio* of Penard, which it occurred to me might possibly be synony-

Digitized by Google

mous. But after further study of the characters of this ameba I began to suspect that my earlier conclusions regarding its specific reference might be mistaken. I accordingly investigated the specific characters of this ameba in connection with some experimental work, under widely varying conditions for about three years, and compared my observations with the published reports of earlier investigators of amebas with the result that I am unable to confirm the specific descriptions by any of the authors named from this ameba.

In the first place, Mereschkowsky's ('79) description is extremely vague (pp. 203-204) Mereschkowsky says the plasm of angulata is transparent, that it contains two kinds of grains: a few large ones and numerous small ones. About three contractile vacuoles and a small round nucleus are present. Few, "am ende zugespitzten (doch nicht wie bei A. *filifera* mit welcher *A. angulata* viel Aehnlichkeit hat) und die gestalt dicker, breiter Kegel habenden, vom Körper ausgehenden Pseudopodien characteristisch. diam. 0.0235." Movements very rapid. The figure illustrating this description is very crude. With the exception of size, this description as far as it goes might apply to a number of species of amebas. The size, 23.5 microns, is very much smaller than that of *bigemma*.

Parona's description of *A. digitata* (1883. Essai d'une Protistologie de la Sardaigne. Arch. des Science physiques et naturelles. T. 10. Troisième periode. p. 225-243. 1 plate) is somewhat more definite than Mereschkowsky's of *angulata*. *A. digitata* possesses a very granular endoplasm, a rounded and conspicuous nucleus, a large contractile vacuole, "pseudopods longs, conique et aigus," pseudopods always in small number. Movement is rather slow. Size, 63 microns (p. 228). The only three characters which may be considered distinctive are the size, the conical and pointed (the figure shows needle points) pseudopods, and the number of contractile vacuoles. None of these characters however are found in *bigemma*. Parona makes no mention of vacuoles in the endoplasm, which, if he had seen a *bigemma*, he could not have helped seeing, since these vacuoles are quite as conspicuous as the nucleus. There can be little question, I think, that Parona described another ameba than *bigemma* under the name *digitata*.

Leidy(79) figures several amebas resembling *bigemma*, *vespertilio* and *digitata* more or less closely, but he regarded them all as varieties of *proteus*, or as forms of uncertain specific reference.

Digitized by Google

In his description of *A. spumosa* Gruber is no more explicit than Mereschkowsky in the instance mentioned. *Spumosa* has broad flat pseudopods, a vesicular nucleus, an endoplasm filled with vacuoles, no granules, a size of 25 microns, according to Gruber. As emended by Penard ('02) *spumosa* possesses these characteristics: A length of from fifty to one hundred and twenty-five microns; form resembling the foot of a goose, with very fine longitudinal lines on the surface; numerous vacuoles; contractile vacuole as much as thirty microns in diameter; a great many bicuspid granules of very small size in the endoplasm; nucleus deformable like that of *A. limax*; a compact nucleolus with a narrow margin of nuclear sap between it and the nuclear membrane. Althought I am inclined to accept the emendation of Penard because of its making for greater definiteness and stability in this difficult genus, yet it appears to me that instead of really emending or elaborating Gruber's description, he actually describes a new and different species. It is evident that another ameba than *bigemma* was under observation by both Gruber and Penard when these authors wrote their descriptions of *A. spumosa.*

Penard's description of *A. verspertilio* (1902, Faune Rhizopodique du bassin du Leman. Geneva, pp. 714) is as follows: size, about seventy microns length; pseudopods have always a conical form, their extremities being usually sharp pointed although the point may be slightly rounded occasionally for a moment[2]; posterior end sticky, dragging debris along as it moves forward; a profusion of extremely small green grains, and sometimes large excretion grains, in the endoplasm; a sperical nucleus with a compact nucleolus which is often covered with fine points; contractile vesicles one, two or three; almost always a considerable number of vacuoles appearing and disappearing as if they were contractile in the endoplasm (pp. 92-95). Penard's figures (p. 94) resemble in a general way the figures of *bigemma*, but in the important points such as the number and character of vacuoles, the shape of the pseudopod extremities, the relative diameters of the nuclear membrane and the nucleus, the character of the "nucleolus," stickiness of the posterior end, inclusions, etc., there is little resemblance between *vespertilio* and *bigemma.*

[2] . . . les pseudopodes ont toujours une forme conique, anguleuse; leur extermite est en principe acérée; mais parfois la pointe peut s'arrondir pour un instant (p. 93). His figures all show sharp needle points on the extremities of the pseudopods.

Digitized by Google

The *Amoeba bigemma* is of medium size, being usually from 100 to 300 microns long in locomotion. Occasionally the size is very much greater. In several old cultures the amebas frequently arrived at a length of 500 microns in locomotion. As a rule, the average size in new cultures is about 150 microns.

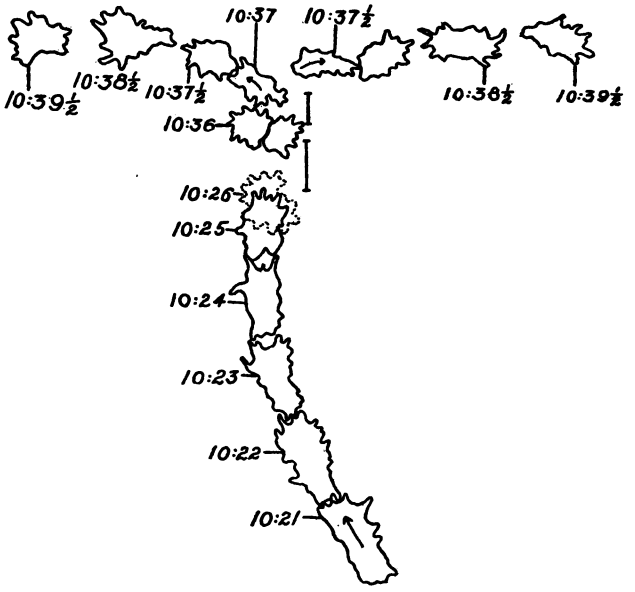


Fig. 1. Camera lucida drawing of an *A. bigemma* in locomotion during the process of fission. The figures indicate the time in hours and minutes. The drawings from 10:37½ on were moved up from their true position the length of the vertical line below; likewise for all drawings from 10:36 on. Note slowing down in the rate of locomotion as the fission crisis was approached, and the gradual increase of speed after division.

Although the general shape of this ameba is subject to very great variation, yet the various transformations are very characteristic. Perhaps the most characteristic feature of these transformations is the tongue like pseudopods, usually short, sometimes very long, which are continually being thrown out at the anterior end and on the free surface during locomotion (Pl. VII, fig. 2). These projections are frequently more or less conical, though more often perhaps they have somewhat the shape

Digitized by Google

of a hart's tongue in outline. These projections are not pseudopods in the same sense that the projections in *dubia* or in *proteus* are pseudopods. In the latter species a pseudopod gives direction to locomotion, that is, the whole ameba frequently flows into a pseudopod. In *bigemma*, however, the whole ameba almost never, if at all, flows into a pseudopod; but the tips of the pseudopods advance at about the same rate of speed as the anterior end of the ameba as a whole advances. The pseudopods are, therefore, to be regarded rather as "toes" on the single pseudopod of which this ameba usually consists. During active locomotion the general shape of the body is most frequently triangular, with the broad base advancing, and more or less compressed. When the animal is disturbed it assumes a spherical shape. When suspended in the water for some time the ameba assumes a shape closely resembling that of the rayed state of a *radiosa*. From a central spherical mass of about thirty or forty microns diameter, long, slender, tapering pseudopods are thrown out which are of a much more permanent character than the pseudopods thrown out during locomotion. These projections are sometimes perfectly straight and of equal size and disposed opposite to each other on the spherical mass. More often however they are curved and of irregular shape and size. Their number is usually from six to eight. All the pseudopods formed during locomotion or while suspended are blunt, very definitely blunt. Of all the thousands of individuals which I have examined in all the different stages and cultural conditions, I have never seen any but definitely obtuse pseudopods. The photographs of live amebas, figs. 4 and 5 indicate the degree of obtuseness characteristic of the pseudopods of this species (Pl. VII).

The streaming of protoplasm during locomotion presents some points of interest. One does not observe the endoplasm flowing slowly in a definite direction in this species as one may in *proteus* or *dubia*, for example, but the streaming is jerky and irregular. The endoplasm seems to drain from the sides and posterior end toward the anterior end against numerous obstructions which often give way. Thus there are developed momentary counter or cross currents and eddies. The peculiar character of the streaming is due to several causes. In the first place the numerous pseudopods are formed and retracted without definite reference to the direction of locomotion. Then again the upper surface of the ameba is not level but extremely irregular; one observes a confusion of high ridges and deep depressions thrown together without

Digitized by Google

observable order. From the depressions more or less permanent pillars of stiffened ectoplasm pass down to the lower surface obstructing the flow of endoplasm. When these pillars give way, as they frequently do, the bottom of the depression is suddenly pushed upward as if the endoplasm were under considerable internal pressure. Another cause of the irregularity of endoplasmic streaming is due to the fact that the anterior end does not advance steadily over the whole front, but by a series of waves here and there. Such a wave consists usually of a web of ectoplasm flowing out between and connecting two or more of the pseudopods; then the web halts momentarily while the pseudopods push out again or while new pseudopods form.

The rear end of the ameba usually plays little part in locomotion in a special way; usually the rear end is smooth and free from any pseudopodial projections. Occasionally, however, a long thin flat pseudopod may be rapidly thrown out near the posterior end which fastens itself to the substratum so well that considerable force is required to dislodge it (fig. 2). The ameba sometimes becomes stretched out to several times its usual length before the pseudopod is pulled loose. This behavior also results often in another phase of movement that I have much more frequently observed in this ameba than in any other, viz., some of the endoplasm begins to flow toward the posterior end until the ameba is cut almost in two in the middle. The connection is so thin that one looks every second for the connecting strand to break, but it does not break. Sooner or later one or the other portion of the endoplasm flows back and the whole mass again becomes unified in streaming.

The rate of movement is rapid, being about 125 microns a minute. Although the anterior end advances very unevenly and irregularly, there are, nevertheless, long segments in the path of an ameba moving on a plane surface that are straight. There is present in this ameba, therefore, the same tendency to keep on moving in the direction in which it started to move as there is in *proteus* (Schaeffer, '12, '18; this point will also be discussed at length in a paper soon to be published).

There is present in this ameba a layer of very thin protoplasm on the outside of the ectoplasm, as usually defined, which moves forward over the ameba in the same direction as that in which the ameba moves, but at a more rapid rate. This is clearly shown by the forward movement over the ameba of small particles which cling to this layer of protoplasm (Schaeffer, '17).

Digitized by Google

During locomotion there is usually a broad zone of clear ectoplasm at the anterior end. Not only are the smaller pseudopods in this region free from granules but the intervening spaces also consist of ectoplasm.

The nucleus is easily seen. Unless obscured by food masses photomicrographs of the living amebas usually show it (fig. 4.). It is spherical or very slightly ovoid (fig. 3). In an ameba of about 200 microns length the nucleus is about twelve microns in diameter. The chromatin consists of very small ovoid granules collected together in the centre of the nucleus in a slightly irregular oval mass of about six or seven microns diameter. The color of the chromatin is a pale bluish yellow green. Between the chromatin mass and the nuclear membrane, which is perfectly transparent, there is a zone of clear nuclear sap. The nucleus is single, though occasionally an ameba is found with two nuclei, which statement is true of course for practically all amebas. The nucleus is deformable though it is not often that one observes striking deformations as it is swept along by the endoplasm.

In one culture of large amebas of this species the nuclei were about twenty-eight microns in diameter, and the chromatin mass of irregularly spherical shape was about fourteen microns in diameter. When these amebas were slightly squeezed under the cover glass one or two masses of mostly perfectly homogeneous pale bluish yellow green material was pressed out from the chromatin mass. There were present here and there large spheres of denser material of the same color as the homogeneous masses. Both the granular mass and the homogeneous masses rounded themselves up and collected near the centre of the nucleus. The material making up the masses seemed to be of the same sort, though I did not employ staining methods to determine whether the interior of the granular chromatin mass was really chromatin or some other substance. It is, however, interesting to know that at least some of the material inside the chromatin mass is not granular while the outside material is.

There seem to be three kinds of vacuoles in this ameba in so far as their functions are concerned. The endoplasm contains scattered about in it everywhere numerous (100 or more) small clear vacuoles of various sizes, mostly under ten microns in diameter, which may be called *permanent* vacuoles. What the function of these vacuoles is, or what conditions are necessary to their origin, remains unknown. That all of these may become contractile is hardly possible, unless they retain their identity in the ameba's body for many hours.

Digitized by Google

The second kind of vacuoles are the *contractile* vacuoles. These have the same general appearance as the permanent vacuoles, excepting that they are more refractive to light. These vacuoles arrive at a diameter of about fifteen microns before they contract. It is very seldom that a larger size is attained. The diastole of these vacuoles is rather slow. The systole is also very slow, occupying from two to six seconds. There are a number of contractile vacuoles present at one time. Under favorable conditions as many as four may be observed to be in the process of diastole at one time. The general appearance of the later stages of a contractile vacuole, that is, higher refractive index of its contents, possibly indicates that these vacuoles are different from the permanent vacuoles from their beginning.

The third kind of vacuole, which may be called the *fecal* vacuoles, are not frequently met with in amebas. These are large spherical vacuoles containing in proportion to their size a very small amount of fecal matter. These may reach a diameter of twenty or twenty-five microns and in occasional large specimens a diameter of from forty to sixty microns. I have not been able to ascertain whether the vacuole originates around the fecal matter or whether after the vacuole is partly formed the fecal matter is voided into it. Since however, the vacuole usually becomes very large, it is evident that the later stages of the increase in size is due to the contained solid matter. The systole of these vacuoles is very slow. The liquid contents is first expelled and then after a pause the solid matter is thrown out in the way common to amebas generally.

None of these different kinds of vacuoles seem to grow by coalescence; at least from extended observation with this point in view I have never seen a single case of coalescence, although vacuoles frequently remain in close contact for a minute or longer.

Another very interesting element in the endoplasm is the crystals. These are usually very numerous and conspicuous, ranging in size from one and one-half to two and one-half microns in length. The general shape is like that of an hour glass or dumb-bell. They seem to be formed of two bicuspid crystals attached to each other by their apices (figs. 2, 3). Under ordinary light they appear dark gray in color; but in polarized light they show up very brightly, and then their twin structure becomes very evident. This is the only ameba known in which such a twin structure of crystal formation is found. The polariscope shows an

Digitized by Google

unmistakable twin structure, however, in only about half the cultures I have so far investigated. In the others the two points of light are joined by a bar of light so that one sees a band of light not constricted in the middle. It may be inferred, however, that in these cultures the earlier stages of crystal formation are also on the twin pattern. It has been found that the character as well as the amount of food influences the size and to some extent the character of the crystals formed. Perhaps the most striking thing about the crystals in this ameba is the fact that they are always attached to the so-called excretion spheres when these are present, as they nearly always are (fig. 3). There is never but one crystal attached to a sphere. The size of the sphere bears no relation to the size of the crystal, the spheres being in some cases just barely visible, while the crystals may be two microns long. When the the spheres are small the crystals are always attached to them at their middle. The sphere and crystal bear a remarkable resemblance to a fish embryo lying on its egg yolk, and they form interesting objects for observation as they tumble along in the streaming endoplasm. Occasional twin crystals are observed apparently free from attachment to spheres, but it is possible that the spheres are extremely minute in such cases, too small to be seen. The excretion spheres rarely exceed a diameter of three microns. In some cultures a few bicuspid crystals with irregular sides are observed occurring singly. The maximum size of these is about two and one-half microns. Occasionally also two twin crystals are found crossed, (fig. 3). Altogether, the crystals form the most definite specific character of this ameba, and the presence of such crystals attached to spheres in an ameba may be regarded as definitely proving its specific identity.

This species is a voracious feeder. Flagellates, shelled rhizopods ciliates, rotifers, nematodes, diatoms, etc., and especially bits of vegetal tissues and masses of bacteria, are readily devoured. The body is frequently stuffed with food.

This ameba is one of the hardiest known to me. I have kept numerous and continuous cultures, after being well established, for several years without much difficulty. The species is subject to very little variation excepting size. In nature this species must be considered rare, though it is found frequently where large masses of vegetation are undergoing decay in quiet water.

Digitized by Google

PELOMYXA LENTISSIMA NOV. SPEC.

Diagnosis. Length in locomotion, 75 to 125 microns. Body usually very much compressed and applied closely to substratum. Changeable in shape, general outline oval with few pseudopods. Quiescent stage with numerous pseudopods of various shapes. Color of body brownish; of protoplasm, pale bluish. Uroid of fine or large root like projections. Rate of movement very slow, from 1 to 2 microns per minute. Nucleus spherical, about 14 microns in diameter. Chromatin in a spherical layer of granules about 11 microns in diameter, with spherical body about 2.5 microns in the centre of the nucleus. Two nuclei frequently present. Contractile vacuoles numerous, 40 or more; maximum size about 10 microns; systole sudden; diastole very slow. Numerous or few small irregular crystalline masses present. Numerous bacterial rods of about 4 microns length present. Only very few refractive (starch) bodies present.

This pelomyxa is readily recognized by its small size and its very slow rate of locomotion (Pl. VIII, fig. 6). It is, in fact, much the slowest moving pelomyxa thus far reported, and I, therefore, propose the specific name of *lentissima* for this species.

I have found this organism in large numbers in old cultures from the marshes of Lonsdale on several occasions. But on account of its small size and its habit of flattening itself out on and sticking close to the surface on which it moves, it more readily escapes detection than other amebas of the same size. The color of this species is a dull brownish, somewhat like that of *P. belevskii*, but not so deep a shade, owing to its smaller size. This color is, of course, due to the contained food materials and the indigestible remains of food objects. It seems to be a habit of this and other pelomyxas to carry for a long time indigestible materials from food objects before excreting them, if indeed some of this material is ever excreted while the animal is in the vegetative stage. The color of the protoplasm is of a bluish violet tint.

This pelomyxa as distinguished from the other species, flattens itself out and sticks very close to the surface during locomotion. At such a time it is thickest in the centre and gradually becomes thinner as the periphery is approached. Around the entire animal there is a clear zone of protoplasm which is hyaline and very thin and of which the exact outer limit is very difficult to see. Pseudopods are continually being extended and retracted from the entire periphery of the animal except from the posterior end. These pseudopods are of clear protoplasm except for a small number of very pale bluish granules which are frequently found at their bases. The pseudopods are broad or narrow and always blunt. They do not usually determine the direction of locomotion.

Digitized by Google

The posterior end terminates in a uroid or group of root-like pseudopodial projections attaching the organism to the substrate (fig. 6). They play a part in locomotion but just what their function is has not been carefully determined. It is certain, however, that the uroid is not necessary to locomotion to the same extent as in *P. schiedti.*

Locomotion in this form is so extremely slow that it is difficult to tell just how it is accomplished. Movement occurs at the rate of from one to two microns a minute, so that it takes from 30 minutes to an hour and a half to move the distance of its own length, or three and one-half months to creep around a baseball. There is a slight but continual and irregular movement of the endoplasm of an oscillatory sort, which, together with slight changes of body shape and the retraction and projection of pseudopods, masks the definite forward movement of the pelomyxa. Figure 9 shows that this movement undoubtedly exists. But under ordinary circumstances it is impossible to detect definite and continuous forward streaming of the endoplasm. It seems as if forward movement was the sum of all the separate local streamings.

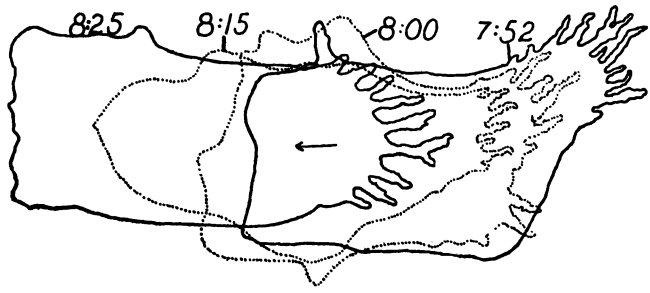


Fig. 9. Camera lucida sketch of a moving *P. lentissima* to show rate of, and form changes during, locomotion.

When suspended in the water or when loosened from the substratum this ameba is remarkable for the number of pseudopods which it throws out from all sides (fig. 7). This habit may indeed be regarded as a distinguishing characteristic of this species while in this stage. The pseudopods are usually more or less conical in shape though they always have blunt ends. The majority are of simple form, others are branched,

Digitized by Google

while some are of very odd shapes. Their length is variable, the maximum being about 50 microns. As might be expected from their rate of locomotion, the transition from a spherical shape in the suspended stage to the locomotive stage consumes much time. As the organism again begins to move, a broad wave of clear ectoplasm appears at some point near the substrate. The wave slowly enlarges as the pseudopods are withdrawn, and gradually the locomotive form reappears.

The nucleus of this species presents several points of interest. In the first place there are usually two. In perhaps three out of four individuals examined two nuclei were found. In no case did I see more than two. It seems that in this species it is the normal condition for a considerable interval of time to supervene between division of the nucleus and the division of the organism. The two processes do not seem to be directly dependent on each other. In the case of most other amebas cell division must closely follow nuclear division, or else in the majority of cases it will become impossible for cell division to take place and the animal in consequence dies (Schaeffer, '16). So that what is normal in division sequences for *Pelomyxa lentissima* is pathologic for *Amoeba proteus*, for example. A *P. lentissima* with two nuclei may be looked upon as an individual whose cytoplasm has not yet divided.

Another point of interest with regard to the nucleus of this species is that a central body is clearly observable in the living condition. The composition of this body has not been investigated. The diameter of this body is from two to two and one-half microns. Its appearance is similar to that of the chromatin granules.

The general appearance of the nucleus is somewhat like that of *P. belevskii*. It is spherical, about fourteen microns in diameter, and contains a spherical layer of chromatin granules of about eleven microns diameter. Not very much can be said about the physical character of the nuclear membrane owing to the very slight movement of the endoplasm. The nuclei are usually found somewhere near the centre of the animal.

The contractile vacuoles are numerous. In one individual there were at least sixty, but whether all were contractile or not could not be determined. The average maximum size of these contractile vacuoles is about ten microns, though many contract when only five microns or so in diameter. Occasionally one may reach fifteen microns before contraction. Consonant with the slow rate of locomotion is that of enlarge-

Digitized by Google

ment of the vacuoles. A vacuole of five or eight microns diameter may take fifteen minutes or longer to enlarge to ten microns, followed by contraction. The systole is, however, relatively rapid, occurring usually in about one second.

The spectroscope shows some very irregular small crystalline masses in the endoplasm sometimes numerous, usually comparatively few. Of "refractive bodies" (starch grains) there are only a few small ones present. Other inclusions in the ectoplasm are the bacterial rods, distinctive of the genus, reaching a length usually of four microns, very rarely of eleven microns; and the very numerous brownish masses of all sizes and shapes so common in several species of pelomyxas. In addition to these bodies there are also numerous very small greenish blue granules found in the endoplasm.

Besides an occasional diatom or shelled rhizopod, I have found no recognizable food bodies in this species.

PELOMYXA SCHIEDTI NOV. SPEC.

Diagnosis. Length in locomotion about 75 microns. Usual shape ovoidal. Color, brownish olive green, almost opaque. Pseudopods very rarely formed. Protoplasm fluid. Movement by eruptive waves of endoplasm partly reflected back along the sides. Rate of movement about 95 microns per minute. Nucleus sometimes single, usually double; spherical, about 7 microns in diameter. Chromatin granules in the form of a hollow sphere immediately under the nuclear membrane or at a slight distance from it. Contractile vacuoles small, numerous; maximum size about 4 microns; diastole rapid; systole instantaneous. Starch grains very numerous, irregular in shape, olive green in color, maximum size about 6 microns. Numerous bacterial rods present of 3 to 4 microns length. Small uroid always present during locomotion.

This species, the smallest of the pelomyxas has been found on several occasions in large numbers in old cultures of material brought from the marshes of Lonsdale (Pl. VIII, fig. 8). Because of its dark color and rapid rate of locomotion it at once attracts attention. Although three or four of my cultures were very rich in this form, it must, nevertheless, be classed as a rare species. The environment must be of a very special kind apparently in order that it may develop in numbers. It remained in my richest culture for about four weeks from the time it was first discovered. I propose for this pelomyxa the specific name *schiedti* in honor of my friend Professor R. C. Schiedt.

Under low magnification this organism appears quite black excepting at a few small places along the sides where the color is temporarily grayish owing to the accidental presence of but few of the starch grains so

Digitized by Google

abundant in this ameba. The posterior end also usually is light gray. Under high powers however, the color is of a dull brownish olive green, due to the brownish endoplasmic inclusions and the numerous starch grains. The protoplasm is bluish green.

This species passes through very slight transformations in shape during locomotion or at other times (fig. 10). Its general shape during locomotion is ovoidal with the anterior end broad, while the posterior end is narrow. Never at any time does the organism flatten itself out to any extent on the substratum. The details of the process of locomotion have not been observed carefully, but the following details may be mentioned. Owing partly perhaps to the fluid nature of the protoplasm, no pseudopods are formed for the purpose of locomotion, but broad eruptive waves of endoplasm break out somewhere near the anterior end into which then flows a part of the animal's endoplasm. These waves are usually partly reflected back along the sides of the animal, leaving a more or less clear space at the farthest point reached by the reflected wave. Occasionally for a short period the pelomyxa may also advance by endoplasmic streaming as is commonly observed in amebas generally, but the larger part of its path is negotiated by eruptive waves as described.

Another important factor in locomotion is the uroid. The animal is not attached to the substratum anywhere except at the uroid. This is readily observed when they are taken up with a capillary pipette. The anterior part of the body is readily displaced by slight currents in the water but the posterior end is not affected in this way. It seems that the thin pseudopodium like projections of which the uroid is formed are for the purpose of holding the organism in place and at the same time allow it to move forward. But just how this is done could not readily be determined owing to the fact that these uroidal projections are very small and very transparent. It has been possible to determine however, that the projections may be thrown out very rapidly, almost instantaneously, so that it is possible that new projections are continually being formed as old ones are being retracted. The alternative view is that the uroidal projections are dragged along over the substratum attaching themselves temporarily and locally as they pass over the substratum. But whatever maybe the exact rôle the uroid plays in locomotion, it is evident that the organism is prevented from rolling over by reason of its attachment to the substratum.

Digitized by Google

Although there is, as has been stated, considerable uncertainty in the direction which the waves of endoplasm take at the anterior end, the path of the organism may nevertheless, because of the activity of its prehensile uroid, be straight for a considerable distance. In a sense therefore the guiding agency in locomotion is located at the posterior end of the ameba.

The rate of locomotion in *schiedti* is rapid, being about 95 microns per minute (fig. 10).

The nucleus of this species presents nothing unusual. Its shape is spherical. The chromatin occurs in rather large masses arranged in the shape of a hollow sphere immediately underneath the nuclear membrane or at some distance from it. Usually two nuclei are present as in the case of *lentissima*. The binucleate condition therefore represents an intermediate stage between nuclear division and cell division. This stage in these two species (*schiedti* and *lentissima*) is much longer than in most typically uninucleate unicellulars, so that the binucleate stage is much more common. These pelomyxas are therefore to be looked upon not as binucleate organisms, but as typically uninucleate. The size of the nucleus is about 7 microns. Owing to the numerous endoplasmic inclusions, the nuclei are difficult to see in the living condition.

The contractile vacuoles are made out only with the greatest difficulty in normal individuals. They are seen only in the small clear areas which are observed occasionally along the sides during locomotion. With very attentive examination the vacuoles may then be seen. The maximum size of the vacuoles is about four microns. Nothing very definite can be said about their number which is certainly not less than ten, but is probably very much greater. The diastole is rather rapid. The systole is practically instantaneous, almost as rapid as the bursting of a bubble on the surface of water. There is a readily observed characteristic rush of protoplasm from the immediate vicinity to the place where the vacuole has just burst, which may be taken advantage of to locate a bursting vacuole without actually seeing the vacuole. In this way it is possible to determine that there are several systoles a minute in different parts of the animal. Doubtless the fluidity of the endoplasm is in some way connected with the small size and sudden contraction of the vacuoles.

This species is full of what are probably glycogen grains (Stolc, A., 1900. Zeit. f. wiss. Zool. Bd. 68). Their color is a shade of olive green.

Digitized by Google

The shapes of the bodies are varied, mostly irregular, angular with rounded corners and edges. The maximum size commonly met with is about six microns. Most of them are only two or three microns long. They are not evenly distributed throughout the body, but there seems to be a tendency for them to collect very near the surface in what is called the ectoplasm. In focussing along the edge one observes a serrated outline, the teeth being represented by the protruding starch grains. I presume that a layer of protoplasm at all times covers these bodies when lying at the surface, though one cannot observe such a layer in the living organism.

Besides the starch bodies there are found considerable numbers of very small spherical bodies of a bluish green color. These are met with in nearly all amebas, but of their nature nothing is definitely known.

The bacterial rods, the presence of which characterizes the genus *Pelomyxa*, are found in considerable numbers in *schiedti*. The length of these rods is about three or four microns.

The number of brownish colored inclusions which are so commonly found in pelomyxas, is small in this species. Sometimes only two or three masses of appreciable size are found. Very little food has been observed in the bodies of these animals. Occasionally a diatom or a flagellate was seen, but in the great majority of individuals no recognizable food objects were found.

When the cultures began to die out, the glycogen bodies began to disappear gradually. In the last few surviving individuals almost no glycogen grains could be seen. The organisms were very pale yellowish and sluggish. Numerous large permanent vacuoles appeared. The nuclei also changed in appearance. The chromatin receded further from the nuclear membrane and collected itself in much larger but fewer granules than normally. From these observations we may conclude that the starch grains are reserve food stores as has been shown by Stolc to be the case in *P. palustris*, and that the cultures died out chiefly because of lack of food.

Zoological Laboratory, University of Tennessee.

Digitized by Google

Fig. 23. Pachytene stage showing the chromsomes pairing by parasynapsis.

Figs. 24 and 25. Diplotene stage showing the beaded appearance and the unfused threads.

Figs. 26 and 27. Late primary spermatocyte prophase showing the condensation of the diplotene threads to form the chromosomes.

Figs. 28, 29, 30, 32, 33 and 35. Side views of primary spermatocyte metaphase, figs. 28, 29, 32 and 35 showing the curved X-chromosome.

Fig. 31. Polar view of a primary spermatocyte metaphase showing ten autosomes and the X-chromosome.

Figs. 34, 36, and 37. Early primary spermatocyte anaphase showing longitudinal division of the autosomes.

Figs. 38 and 39. Parts of primary spermatocyte anaphases showing the curved X-chromosome going to one pole.

Figs. 40 and 41. Early primary spermatocyte telophase.

Figs. 42, 43 and 44. Late primary spermatocyte telophase.

Figs. 45, 46 and 47. Secondary spermatocyte prophase.

Figs. 48 and 50. Secondary spermatocyte metaphase showing ten autosomes plus the X-chromosome. Fig. 50 from a smear preparation.

Fig. 49. Secondary spermatocyte metaphase showing ten autosomes.

Fig. 51. Early secondary spermatocyte anaphase showing equational division. From a smear preparation.

PLATE X

Figs. 52 and 53. Early secondary spermatocyte anaphase showing equational division.

Fig. 54. Secondary spermatocyte telophase showing approximately ten autosomes plus the X-chromosome at each pole.

Fig. 55. Same as fig. 54 except that the X-chromosome is not present.

Figs. 56 and 57. Spermatid showing the spermatosphere with the centrosome imbedded in it and the remnants of the secondary spermatocyte spindle (idiozome).

Figs. 58, 59, 60 and 61. Spermatids showing the formation of the acrosome from the idiozome, the migration of the spermatosphere plus the centrosome to the other side of the nucleus, and the division of the centrosome.

Figs. 62, 63 and 64. Spermatids showing the migration of the nucleus to one side of the cell, the formation of the middle piece, end knob, posterior centrosome and the axial filament.

Fig 65. Immature sperm which has cast off its cytoplasm except that destined to become the sheath of the middle piece and the fibre which seems to connect it with the Sertoli cell.

Fig. 66. Mature sperm viewed from the broad side.

Fig. 67. Mature sperm viewed from the side.

Fig. 68. Cross section of a tubule the sperm cells of which have all matured leaving only a few Sertoli cells and fibres.

Fig. 69. Polar view of a somatic cell of a female foetus showing 22 chromosomes.

Figs. 70 and 71. Anomalies showing heterogenic division.

Figs. 72 and 74. Giant cells showing more than one nucleus and their associated spermatospheres.

Fig. 73. Two spermatids side by side.

Digitized by Google

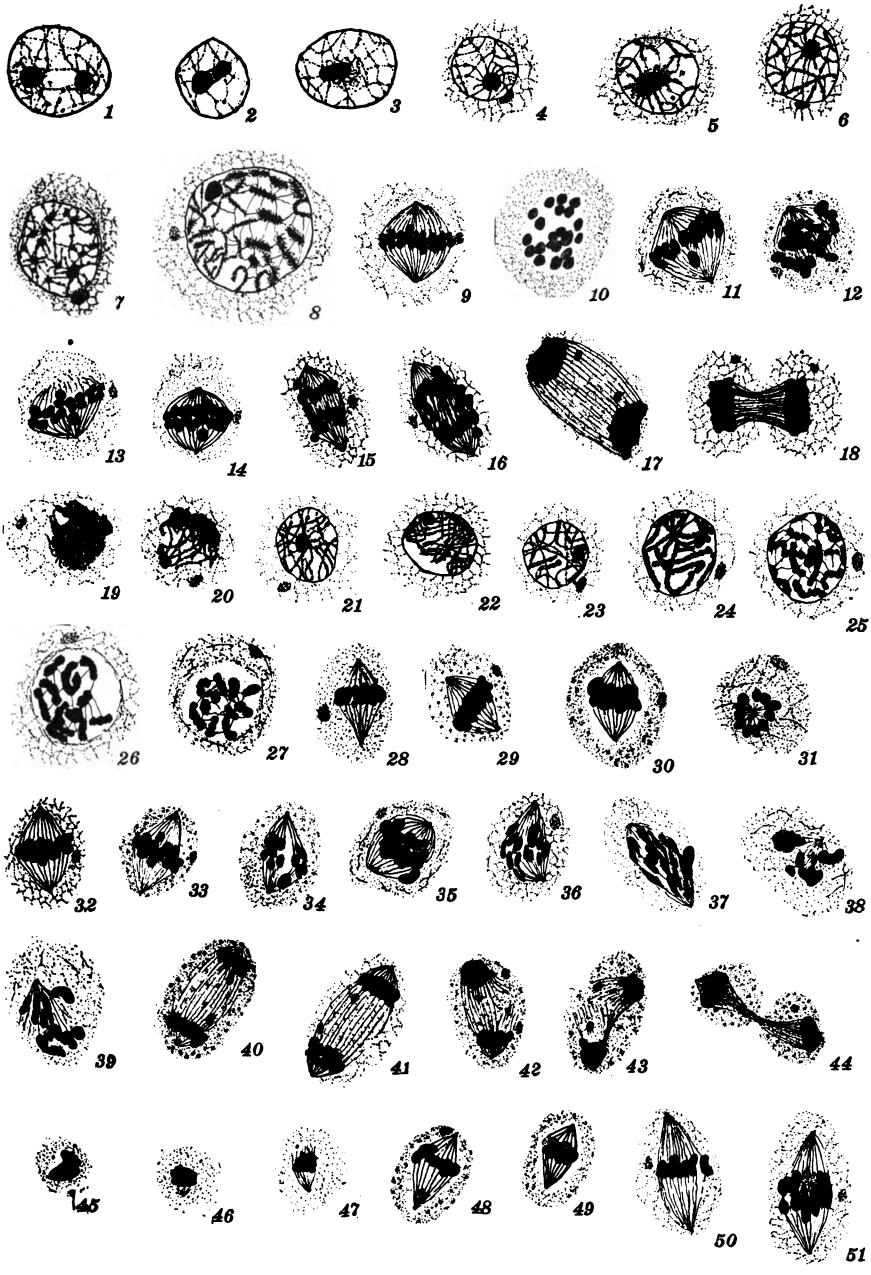


PLATE IX

MALONE

Digitized by Google

Digitized by Google

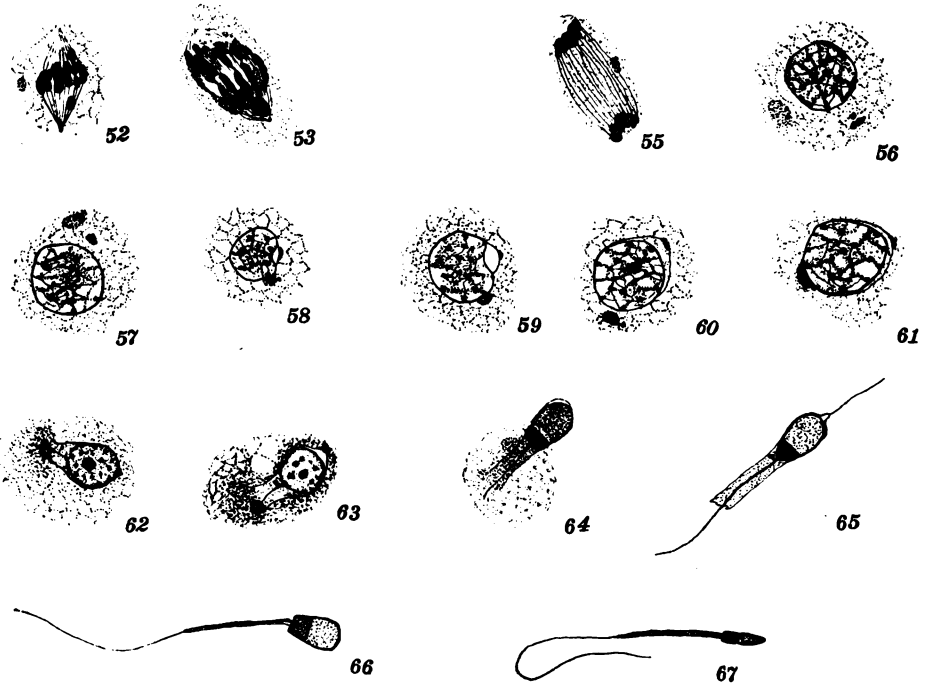


PLATE X

MALONE

Digitized by Google

Digitized by Google

THIGMOTACTIC REACTIONS OF THE FRESH WATER TURBELLARIAN, *PHAGOCATA GRACILIS*, LEIDY

BY

BERNOL R. WEIMER

The Turbellaria have ever been popular subjects for experimentation, yet in looking over the literature, I find that very little has been done upon the thigmotactic reactions of these animals. Thinking that here was a problem that might yield some interesting results, I collected a number of the animals in Falling Run, Morgantown, West Virginia, and these were used in the following experiments. Five specimens were sent to Dr. H. S. Pratt of Haverford College, and were identified by him as Phagocata gracilis.

Six rocks of varying degrees of roughness were collected and cemented together with Portland cement in a circular glass dish 7 cm. high. The rocks were so arranged as to form a surface as nearly flat as possible. The outer walls of the dish were covered with heavy black paper in order that light could fall only vertically upon the surface of the rocks.

After allowing water, which was changed frequently, to stand in the dish for three weeks, some animals were placed in the dish. All the animals were in good condition, but those animals which came in contact with the cement between the rocks, secreted a slimy substance and died. The cement was then coated with paraffin, and at the same time a piece of glass of nearly the same area as the different rock surfaces, was waxed in.

After carefully washing the paraffin for two days in running water, some animals were again placed in the dish and after two days were alive and in good condition. Forty animals were then put into the dish which was placed beneath the skylight in a room so darkened that all the light came from this source. The surface of the rocks in the experiments was covered by water to an average depth of 1.5 cm. The temperature of the water varied from 21° -25° C.

In collecting the rocks used in the experiments, care was taken that the surface should have different degrees of roughness and that each rough surface should be as nearly uniform as possible. The rocks and the glass plate were each numbered with India ink. These numbers are used in the tables given, to designate the rock and, obviously, the surface. Rocks numbered 1, 2, 3, and 4 were of weathered sandstone and rock number 5 of weathered lime-stone.

Digitized by Google

The surface of rock number one was composed of rounded quartz crystals of about the same coarseness as granulated sugar. Rock number two had a surface somewhat smoother than number one, the particles of sand were about as coarse as table salt. Rocks numbered four, three and six approached, in the order mentioned, rock number five, a smooth lime stone. Thus the rock surfaces varied in coarseness or roughness of surface from particles the size of granulated sugar to a comparatively smooth surface in the following order: one, two, four, three, six, and five. For a surface a grade smoother than the rocks described, there was the glass plate and the paraffin coating the cement. Practically the only surface difference noticed between the glass and the paraffin was, that the paraffin had a slightly greasy feeling.

The following explanation refers to tables 1 and 2. The total number of animals counted on a given surface during a set of observations was found and this number, divided by the number ofobservations in the set, gave the average observation for the set. This average observation for each set was added and the average of this total gave the average observation for all the sets. Since only one set of observations was taken in tables 3 and 4, the second average of the sets, was not necessary. The area of the paraffin and the vertical glass sides of the dish covered by water were averaged as one surface (para-glass in tables). This para-glass surface was used as the common unit area and from this the relative number of animals resting on a common unit surface, was computed. Since the paraffin and the vertical glass surface was so nearly alike, both were averaged as one. With this brief explanation, the tables given should not be difficult to understand.

The data set forth in table 1 was procured in the following way. The observations were taken at either ten or fifteen minute intervals and usually after each observation the animals were stimulated, i.e. caused to move from the place they were resting. No distinction was made between an animal moving across and one at rest on a surface. This probably accounts for the high averages for the various surfaces in table 1. The dish was frequently moved around to counteract any light reaction, tho the light apparently made no difference since it came vertically from above. A tendency to gather in groups was also noticed. These groups were usually found on the paraffin. The animals in these groups were always stimulated after an observation.

Digitized by Google

TABLE 1

No. of lbs.	10	10	8	10	8	7	15	Total 68		
	av. set1	av. set2	av. set3	av. set4	av. set5	av. set6	av. set7	av. of all obs.	No. animals per unit surface	Total no. of animals counted
Rock										
1	1.70	1.80	2.75	0.70	2.00	4.30	1.60	2.13	25.53	145
2	2.40	1.20	1.50	0.40	2.55	3.66	0.46	1.73	15.65	108
3	2.10	3.40	2.70	1.10	2.44	2.50	1.05	2.19	18.79	249
4	0.90	4.80	8.25	0.50	2.20	1.16	1.90	2.81	34.59	192
5	2.30	2.40	1.66	0.80	1.60	2.30	1.90	1.85	15.17	126
6	1.40	1.20	1.40	1.30	1.70	3.60	1.26	1.66	17.66	113
Glass 7	1.70	0.90	1.00	0.70	1.30	2.10	1.20	1.27	16.25	86
Para-glass	32.70	28.50	25.12	34.50	28.30	30.30	31.86	30.18	30.18	2053

Observations showing number of animals seen on each surface with vertical illumination only. The time between sets varied from a day to a week. The surfaces in order from roughest to smoothest are as follows: one, two, four, three, six, five and glass.

From table 1 it is seen that when all the surfaces have been reduced to the same unit area, the three highest averages of animals resting, are as follows, rock four, 34.59, paraffin-glass, 30.18 and rock one 25.53. The other surfaces have much lower averages and do not vary much among themselves. One peculiar fact to be noticed is that rock one has the roughest surface, the glass-paraffin has the smoothest surface, and intermediate between these two is the surface of rock four. It would be well to remember that after each observation the animals were stimulated. Since so few animals were found resting, they were averaged with those moving.

The data in table 2 differs from that found in table 1, in that all the observations were taken in a photographic dark room. An electric light was used only long enough to record the observation. The observations were taken at intervals varying from 30 minutes to an hour and the animals moved from their resting position after each observation. As a result of the longer interval elapsing, most of the animals were found resting when the observation was recorded.

Digitized by Google

TABLE 2

No. of obs.	4	6	6	4	Total 20		
	av. set 1	av. set 2	av. set 3	av. set 4	av. of all obs.	No. of animals per unit surface	Total no. of animals counted
Rock 1	0.00	1.50	1.60	0.75	.96	11.41	192
2	2.00	3.00	1.16	0.25	1.60	14.38	32.00
3	1.00	2.33	0.80	2.50	1.65	14.15	33.00
4	1.50	1.50	0.16	1.00	1.04	12.80	21.00
5	0.25	1.66	6.10	1.00	2.22	18.20	45.00
6	1.50	0.50	3.00	3.75	2.18	23.06	44.00
Glass 7	1.50	2.30	1.50	0.25	1.38	17.66	28.00
Para-glass	32.75	25.60	27.60	30.75	29.17	29.17	584

Observations taken in photographic dark room showing number of animals counted on each surface. The time between sets varied from a day to a week.

The surfaces in order from roughest to smoothest are as follows: one, two, four, three, six, five and glass.

The averages of these observations in table 2 show, that when all the surfaces have been reduced to the same unit area, the surfaces having the most animals resting upon them are the paraffin-glass, 29.17, and rock six, 23.06. Rock six has a comparatively smooth surface of weathered lime-stone. The averages for the other surfaces are about the same.

After conducting the first set of experiments, the results of which are found in tables 1 and 2, the glass was broken from around the cement block containing the rocks. The cement necessarily exposed was covered with a coating of bees wax. The whole block, surface downward, was then placed upon three glass supports, 2.5 centimeters high, in an enameled pan. The pan was then filled with water until it covered the surface of the rocks. This apparatus was first put in a photographic dark room, and observations taken each hour by lifting the rocks. The results were so decidedly negative that only a few observations were taken. See table 3. The animals not only did not rest upon the rock surfaces but were found scattered in average numbers over the entire

Digitized by Google

bottom and sides of the pan. Only an average number was found on the bottom of the pan underneath the rocks.

TABLE 3

Surface	Observations					Av.	No. animals per unit surface	Total no. animals counted
Rock 1						Av.		
2	1					0.20	1.19	1
3		2				0.40	3.43	2
4								
5								
6								
Glass								
Para.								
Pan	39	38	40	40	40	39.90	39.90	237

One set of five observations taken in photographic dark room with the rocks inverted, showing the number of animals counted on each surface.

The surfaces in order from roughest to smoothest are as follows: one, two, four, three, six, five and glass.

TABLE 4

Surface	Observations									Av.	No. animal per unit surface	Total no. animals counted
Rock 1												
2									1	0.09	0.80	1
3												
4												
5									1	0.09	0.73	1
6												
Glass												
Para.					1		1			0.18	0.18	2
Pan	40	40	40	40	39	40	39	40	38	39.5	39.50	435

One set of nine observations taken with vertical illumination only and with rocks inverted, showing the number of animals counted on each surface.

The surfaces in order from roughest to smoothest are as follows: one, two, four, three, six, five and glass.

Digitized by Google

The same apparatus was then placed underneath the skylight in a room so darkened that all the light came from this source. The same negative results were obtained as in the photographic dark room, though the difference was noted in that the animals in this experiment, gathered on the pan underneath the rocks, whereas in the dark room this preference was not shown.

According to Pearl ('02), the ventral surface of Planaria maculata is strongly positively thigmotactic; but this does not explain the tendency of the animals to rest in angles. This resting in angles he calls goniotaxis. The physiological condition of reduced tonus helps to determine whether or not the animal will rest on a smooth surface.

The increased resistance to movement may be one cause for stopping on the uneven surface tho the most important factor is probably light. Nevertheless, instances are known where the animal may stop in a bright light, tho such instances are rare.

From table 1 it is seen that rock four has an average of 34.59 animals per unit area. The surface of this rock in degree of roughness, is midway between the rough granular surface of rock number one (average 25.53) and paraffin-glass (average 30.18). Table 2 shows that the paraffin-glass surface (average 29.17) is the highest per unit area, followed by rock six (average 23.06) whose surface is of smooth weathered limestone. In tables 1 and 2, it will be noticed that surface number seven is of glass. This surface is horizontal, not vertical as is that averaged with the paraffin. If the average of this surface (16.25 in table 1 and 17.66 in table 2) were added to the surface average of the paraffin-glass (average 30.18 in table 1 and 29.17 in table 2), the total average of these surfaces would be 46.43 in table 1 and 46.83 in table 2. This is much higher than any of the rough surfaces. From this the conclusion may be drawn that the animals prefer a smooth surface.

Pearl ('02), in describing the method of locomotion of Planaria maculata says that the ventral surface of the body constantly secretes mucus in greater and lesser quanities. This is very sticky and under normal conditions adheres to the surface on which the animal reposes. Thus between the animal and the surface on which it moves there will be a constant layer of mucus. The beating of cilia in this mucus pushes the animal forward. Considering this explanation, then, more mucus may be secreted by an animal when passing over a rough surface than when passing over one that is smooth. Likewise a regular granular surface

Digitized by Google

might not have quite so much effect as a very angular surface. Pearl ('02), also suggests that the reduced physiological tonus of the animal might cause it to rest on the rough surface.

To find whether or not more mucus was extruded and secreted when passing over a rough surface than over a smooth one, the following experiment was tried. A piece of ordinary window glass was scratched or grooved by a diamond glass cutter. The glass with the grooved surface was then placed in a large petri dish and covered with water. Some of the animals were then placed in the dish and allowed to move over the grooved surface. After an hour the animals were removed and the glass "developed" by immersing in Delafields' hematoxylin, which stained the slime blue. This gave better results than the method used by Pearl ('02), who used a solution of carmine. This was tried and the carmine particles by adhering to the mucus, showed the tracks but the mucus was not stained and hence a close study of it could not be made. The glass was then broken so that cross sections of the grooves could be examined with the microscope. A number of sections were thus examined but at no place could a greater slime secretion be noticed where the slime tracks crossed a groove.

Thinking perhaps that the width and depth of the grooves in a surface might cause some difference in the amount of slime secreted, the bottom of a large petri dish was covered by a layer of paraffin to an average depth of 5 mm. In this paraffin were cut grooves varying from 0.20 to 3 mm. in width and the same in depth. The dish was then filled with water and in it were placed some animals. The smaller grooves did not seem to reduce the rate of locomotion of the animals. However, when an animal approached one of the wider grooves, it would hesitate, raise up the anterior end of the body and move it to and fro, then pass across without touching the bottom of the groove.

After the animals had been undisturbed for two hours, they were removed and the water replaced by a solution of Delafields' hematoxylin. On examination of the stained slime tracks it was found that in few cases was there a greater amount of slime secreted. The slime strands did not descend to the bottom of the grooves but in most cases were continued on across as a sort of bridge. In some cases these strands were broken but even then no abnormal amount of slime was found. At the edges of the grooves, however, there were places where there seemed to be a greater amount of slime. These were the places, probably, where the animals hesitated.

Digitized by Google

From these results it would appear that there is the same amount of slime on all surfaces and that no abnormal secretion is caused by a rough surface. Thus a change in the physiological condition of the animal as caused by the difference in mucus secretion, would not effect the thigmotactic reaction to surfaces of varying roughness and smoothness.

Pearl ('02), suggests that perhaps the rough surface offers more resistance to movement than does a smooth surface. This naturally would cause a slower rate of locomotion when an animal was passing over a rough than when passing over a smooth surface but since an equal amount of slime is secreted on all surfaces, there should be approximately the same rate for all surfaces. Accordingly the rate of locomotion was found for two surfaces, the one of rough sandstone, somewhat coarser than rock one, and the other glass.

A piece of white paper was marked off in 1 cm. squares and placed underneath a large petri dish filled with water. The surface of the rock was marked off in 1 cm. squares with waterproof India ink and around the edges were placed sides of pasteboard to keep the animals from wandering off the surface. The rock was placed in a galvanized pan which was filled with water to such a height that the surface of the rock was covered to a depth of 2 cm. The two surfaces were placed underneath the skylight in a room from which all other sources of light were cut off.

The four animals used in these experiments were kept in separate dishes carefully marked. When the animals were transferred from these dishes to the surfaces, a camel's hair brush was used in order not to injure them. In the course of these experiments, animal number one died, so that most of the tables show the results of the reactions of numbers two, three and four, all of which were active, healthy, individuals. The animals were placed on the surface singly, and a number of observations taken by means of a stop watch, as to the length of time required to move over 1 cm. of surface. No allowance was made for an approximate deviation of 2 mm. from a straight line in passing over 1 cm. of surface.

The data recorded in table 5 was procured thus. A number of observations of the rate of locomotion of each animal was taken on two consecutive days for the glass surface and on the two following days for the rock.

Digitized by Google

TABLE 5

Animal	Rock 1st day		Rock 2nd day		Av. rate in mm. per sec.	Glass 1st day		Glass 2nd day		Av. rate in mm. per sec.
	no. of obs.	av. rate in mm.	no. of obs.	av. rate in mm.		no. of obs.	av. rate in mm.	no. of obs.	av. rate in mm.	
1	15	0.92	15	1.23	1.07	10	0.74			0.74
2	15	0.80	15	1.10	0.95	16	1.43	18	1.2	1.31
3	15	1.05	15	1.17	1.11	17	1.47	18	1.46	1.46
4	15	0.97	15	1.24	1.10	18	1.38	18	1.47	1.42

Observations showing the rate of locomotion. Observations taken on one surface only each day

Table 5 shows that the average rates per second of animals number three and four are the highest, being 1.11 mm. and 1.1 mm. on the rock and 1.46 mm. and 1.42 mm. on glass. This shows a difference in rate of speed per second between the glass and the rock of 0.35 mm. and 0.32 mm. Animal number two shows a difference per second of .36 mm. In these three instances the animals moved more rapidly on the glass. However, a further examination shows that an animal in twenty-four hours time will vary in rate of locomotion on the rough surface .31 mm. and on the smooth .21 mm. per second. Walter ('02), who tested the rate of speed on glass only, found that the rate varied from 1.22 mm. to .96 per second. So the variation in rate between the two surfaces can scarcely be attributed wholly if at all, to the difference between the two surfaces but to some other factor. This is further proven by a study of tables 6 and 7.

The data in tables 6 and 7 differs from that in table 5 somewhat. A number of observations on the rate of locomotion of an animal was taken first on one surface and immediately on the other. Several hours later another set of observations was taken. This lessened the chance for so great a change physiologically as to effect the speed. The results are seen in tables 6 and 7.

Table 6 shows at 8:00 the maximum difference of speed of all the animals, between the two surfaces to be .16 mm. per second. At 2:00 the maximum difference is .1 mm. per second.

Digitized by Google

TABLE 6

Animal	time	Rock		Glass	
		no. of obs.	av. rate in mm. per sec.	no. of obs.	av. rate in mm. per sec.
2	8:30	8	1.15	7	1.21
3	until	7	0.98	7	0.92
4	11:00	9	1.17	7	1.01
2	2:00	7	0.99	9	0.99
3	until	7	1.02	9	1.12
4	4:00	7	1.01	5	1.11

Observations showing rate of locomotion. All the observations were taken on one day.

TABLE 7

Animal	time	Rock		Glass	
		no of obs.	av. rate in mm. per sec.	no. of obs.	av. rate in mm. per sec.
2	9:00	5	1.06	7	1.36
3	until	7	1.01	8	0.98
4	11:00	7	1.19	8	1.24
2	1:30	5	1.03	7	1.12
3	until	7	1.01	7	1.17
4	3:00	7	1.19	4	1.33

Observation showing rate of locomotion. All the observations were taken on one day.

Table 7, a day later, shows the maximum difference in rate at nine o'clock to be .3 mm. per second and at 1:30 o'clock to be .16 mm. per second. Further, in some cases the rate of speed is greater on the rock than on the glass.

The difference, then, in rate of locomotion between a smooth surface and a rough surface is so small as to be almost negligible. This further

Digitized by Google

shows that the amount of slime extruded and secreted is not increased for a rough surface since this would probably reduce the speed of the animal.

According to Pearl, ('02), the method of locomotion in Planaria maculata is by means of the cilia beating in the mucus strands. Thinking that perhaps a study of the cilia and hypodermis might throw some light upon the thigmotaxis and locomotion of these animals, some work was done upon the histological structure of Phagocata gracilis.

Planarian tissue is one of the most difficult of animal tissues to study because it is so hard to fix properly and to stain. A fixative may give good results in one case and not in another. Five different fixing fluids were tried, hot Gilson's, hot and cold corrosive-acetic, twenty-five per cent solution of nitric acid, hot corrosive sublimate and a fixative recommended by Woodworth, ('91), made up of a saturated solution of corrosive sublimate in fifty percent nitric acid; of these, corrosive sublimate gave the best results. Of the various stains, borax-carmine, picrocarmine, neutral red, picric acid and carmine (aqueous), Delafields hematoxylin, Ehrlich's hematoxylin and eosin gave the best results. The sections were cut 6.6 microns thick. In all about thirty animals were studied.

The study was made principally of the hypodermis. On the ventral side, figure 2, it is made up of strongly ciliated columnar epithelial cells These contain large subcircular nuclei, nu, with evenly distributed chromatin granules. Definite cell walls between the cells could not be distinguished. Between the cells are spindle shaped, homogeneous bodies, the rhabditi, rb, which (Woodworth, '91) are developed in subcutaneous flask-shaped cells which are ectodermic in origin. These were supposed to be homologous to the nematocysts of the Coelenterata but later were supposed to be gland secretions. Underneath the hypodermis is a homogeneous layer, the basement membrane, bm. Under this is found the different muscle layers and the body parenchyma, figure 1.

The dorsal hypodermis, figure 3, is made up of columnar epithelial cells somewhat longer than those on the ventral surface. No cilia were found on these cells except near the edges of the animal. The rhabditi, rb, were much larger and more numerous than on the ventral hypodermis. They were found in groups of two or three. So thick were these that the nuclei of the cells, in many cases, were pressed out of shape and often concealed. The nuceli, nu, of the cells, when seen, appeared to have

Digitized by Google

the same structure as those in the ventral side. Beneath the cell layer is found the basement membrane, bm. It is somewhat wider than on the ventral surface but has the same appearance. Beneath this membrane are the muscle layers and the parenchyma, mus. par. Imbedded in the parenchyma are numerous rhabditi mother cells, rb. mc, figure 1.

According to Woodworth, ('91) these rhabditi rapidly disintergrate when extruded into the water to form a slime which, by entangling the prey, aids the animal in procuring food. They may also be used for protection. Whether or not these rhabditi play any part in the locomotion of the animal, I was unable to determine.

In reviewing the literature on planaria there seems to be a difference of opinion among investigators regarding the distribution of cilia on the surface of the animal. Pearl ('02) finds none on the dorsal surface of Planaria maculata. Metschinikow ('66) and Kennel, ('79), found cilia covering the whole surface of Rhynchodesmus and Geodesmus but Zacharias, ('88), states that the dorsal surface of a variety of Goedesmus is bare. Vejdowsky, ('90), maintains the same for Microplana, the cilia in the latter cases being confined to the ventral surface or sole. Woodworth, ('91), found the cilia on Phagocata gracilis (collected near Cambridge, Mass.) to cover the entire surface of the body. I was able, tho I used the same technique as Woodworth, to find cilia only on the ventral surface and sides of the animal. However, these animals were collected in Falling Run, Morgantown, W. Va. This difference in localities may account for the variation in ciliation. Mosely, '74, explains that this absence of cilia on the dorsal surface of Bipalium may be due to the fact that the cilia on the dorsal surface of land planarians are weaker thru comparative lack of function and are more easily destroyed by reagents. However this is still an undecided question.

Summary

The results in tables 1 and 2 show that there is a preference shown for a smooth surface rather than a rough one.

Tables 3 and 4, are interesting in that they substantiate the results found by Olmstead, ('17), that unfed Planaria maculata are positively geotropic, that is to say, have a tendency to keep the ventral side toward the stimulus of gravity. Likewise these results show that *Phagocata gracilis* is strongly negatively phototropic.

Digitized by Google

Since no mucus was found filling the grooves and cracks in the glass, it is clear that there must be no abnormal secretion of mucus and this is further strengthened by the results found in tables 5, 6 and 7, that there is an almost constant rate of locomotion on both a smooth and a rough surface. The last three tables also show that there is a variation in rate of locomotion which may be due to the different physiological states of the animal.

A histological study of the hypodermis discloses the fact that no cilia could be found on the dorsal side of the animal, which may be due to a difference in the variety of the variety of the animals, since Woodworth, ('91), found cilia on the dorsal hypodermis of the same species, collected in New England.

Conclusions

1. Phagocata gracilis is positively thigmotactic to a smooth surface.
2. This is not due to varying amounts of mucus secreted.
3. Phagocata gracilis is positively geotropic and strongly negatively phototropic.
4. The rate of locomotion is the same for both a rough and am sooth surface.
5. No cilia are found on the dorsal surface of Phagocata gracilis collected at Morgantown, W. Va.

University of W. Va.
Morgantown, W. Va.

References

Olmstead, J. M. D.
1917. Geotropism in Planaria maculata. Jour. Animal Behavior, Vol. VII, pp. 81-86.

Pearl, R.
1902. The Movements and Reactions of Fresh Water Planarians. Quart. Jour. Micr. Science, Vol. XLVI, pp. 508-714.

Walter, H. E.
1908. The Reactions of Planarians to Light. Jour. Exp. Zool., Vol. V, pp. 35-162.

Woodsworth, W. McM.
1891. Contributions to the Morphology of Turbellaria I. on the Structure of Phagocata gracilis, Leidy. Harvard Mus. Comp. Zool., Vol. XVI, No. 1, pp. 1-42.

Digitized by Google

Explanation of Plate

Plate XI

Fig. 1. Transverse section of the animal anterior to the mouth, × 22.
Fig. 2. Portion of the ventral hypodermis, × 2000, approximately.
Fig. 3 Portion of dorsal hypodermis, × 2000, approximately.

Lettering

bm. basement membrane
cil. cilia
c. mus. circular muscle
hyp. hypodermis
l. cil. limit of cilia
l. mus. longitudinal muscle
mus. muscle
mus. par. muscle and parenchyma
nu. nucleus
par. parenchyma
ph. pharynx
rb. rhabite
rb. mc. rhabdite mother-cell

Digitized by Google

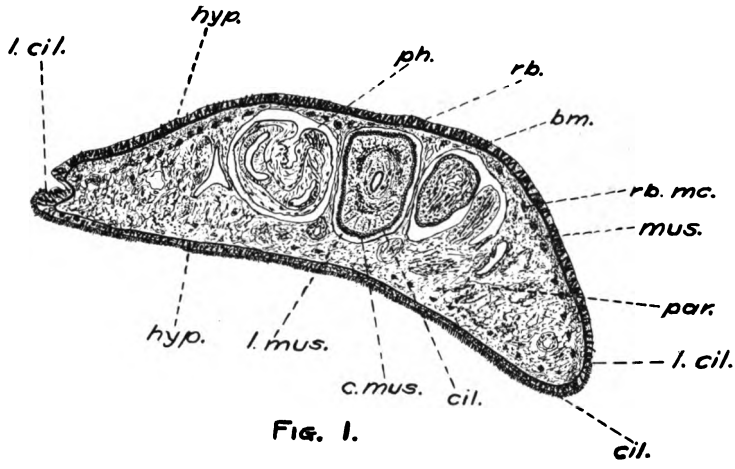


Fig. 1.

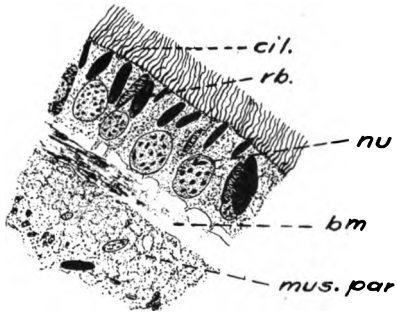


Fig. 2.

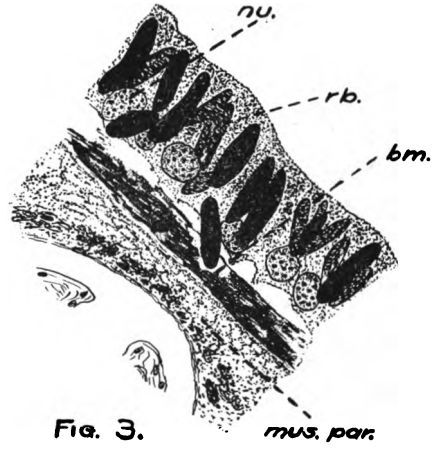


Fig. 3.

PLATE XI

WEIMER

Digitized by Google

Digitized by Google

ADDITIONS TO OUR KNOWLEDGE OF *UNIONICOLA ACULEATA* (KOENIKE)*

BY

ERNEST CARROLL FAUST

The material of *Unionicola aculeata* (Koenike) on which this paper is based was secured from one of many specimens of *Lampsilis luteola* Lamarck, from North Judson, Indiana. The host was determined by Mr. Frank C. Baker, Custodian of the Natural History Museum of the University of Illinois. The general organization of the mite conforms to the type species described by Koenike (1890), but certain interesting variations and certain structures yet inadequately described deserve to be made a matter of record.

U. aculeata was originally described from Germany by Koenike, as similar in many respects to *U. crassipes* (Müll.) and in others to *U. figuralis* (Koch). It differs from the former in the exact structure of the palpus, in the number and distribution of the genital acetabula, and in the structure of the chitinous ovipositor. Wolcott (1899) describes this species from Michigan. The mite recorded by Soar (1899) as *Atax taverneri* has also proved to be *U. aculeata*. Piersig (1901) has made two subspecies of *U. aculeata*, separating the American form, *U. aculeata sayi*, from the European form, *U. aculeata aculeata*, on the basis of the number of the tarsal claws, comparative lengths of the leg segments, exact relations of the parts of the genital field, and a slight size difference.

The specimens collected by the writer conform to those described by Wolcott in most respects. In size they belong to Piersig's smaller group, since the female measures about 680μ long and the male measures about 640 μ long.

Wolcott's specimens have a terminal segment to the female palpus which is somewhat attenuate distad, ending in four small claws. In the writer's material the palpus has a more distinctly thickened terminal joint, with two finger-like claws and two thumb-like claws in the female and with two finger-like claws and only one thumb-like claw in the male. The penultimate joint of the palpus in Wolcott's material has a needle spine which is not present in the North Judson material. In the female of this material the basal segment is in each case the thickest of the six.

*Contributions from the Zoological Laboratory ot the University of Illinois under the direction of Henry B. Ward. No. 111.

Digitized by Google

The joints are progressively narrower and longer from base to tip. In the female there are three conspicuous spurs on the ventral side of the basal joint, and two undivided sickle-shaped claws at the end of each tarsus. Long needle spines are prominent on segments two to four. Soar (1899) figures the claws of the first leg as cleft, a feature which Wolcott (1899) describes for both *U. aculeata* and *U. crassipes*, while those of the writer's material are entire as in legs two to four.

Perhaps the most interesting feature of the entire body structure of *U. aculeata* is the heteromorphic fourth leg of the male. On this appendage both the joints and spines are curiously modified so that they present a striking ornate appearance (fig. 5). The tarsus is a long attenuate flat plate as in the female, with two undivided terminal claws and two accessory spines. The main shaft of the tarsus is free from spines. The tibia is short and thick with seven needle spines, two large heavy spines and one small spine. The third segment has three large and three small blunt spines, in two parallel lines at the outer edge of the joint. The fourth and fifth joints are both shorter than joint three altho not as short as the tibia. They are both supplied with several short bristles. The basal joint is irregularly sculptured and bears four spines.

The genital field of the female resembles closely that described by Wolcott, altho the cleft between the two plates is not distinguishable as a separate structure and no posterior papillae are found in the writer's material. The external male genital organs (fig. 3) are very complicated, consisting of an ornate sculpture of chitin, to which prominent muscle bands are attached.

A thoro study of the new material and comparison with that described by Koenike, Wolcott, and Soar, shows the wide range of variability within the species, while at the same time it discredits Piersig's separation of the species into two subspecies. It seems much more desirable to regard the species simply as highly variable rather than to maintain a subdivision, since the lesser similarities and differences grade into each other almost imperceptibly in various specimens.

Unionicola aculeata has been credited by some as a parasite of the Unionidae and by others as free-swimming. Koenike (1915) has shown that it is free-swimming during a considerable part of its life and seeks the mussel at times of metamorphosis and propagation. This fixes our knowledge of the periodicity of the parasitism, but leaves us in the dark with regard to the degree of parasitism. The mites described in this

Digitized by Google

paper were found embedded in the subdermal connective tissue of the mantle and foot of the mussel (fig. 6). Their position was related to no definite axis of the host. Around it was a thin tissue cyst. In no case were they found to have injured the host outside of the cyst. Thus, altho an endoparasite, the evidence shows it to be only a temporary lodger.

University of Illinois.

LITERATURE CITED

KOENIKE, F.
1890. Ein neuer Bivalven-Parasit. Zool. Anz., 13:138-140.
1915. Beitrag zur Kenntnis der Wassermilbe *Unionicola aculeata* (Koen.). Arch. Hydrobiol. Planktonk., 10:308-319, 1 Taf.

PIERSIG, R.
1901. Hydrachnidae. Das Tierreich (Schulze). Lief. 13. 336 pp., 87 figs.

SOAR, C. D.
1899. Atax Taverneri sp. nov.? Jour. Quekett Micr. Club, 7(2):219-221, 1 pl.

WOLCOTT, R. H.
1899. On the North American Species of the Genus Atax (Fabr.) Bruz. Trans. Am. Micr. Soc., 20:193-259, 5 pl.

Digitized by Google

Explanation of Plate XII

Fig. 1. Dorsal view of female palpus, X 226; fig. 2, ventral view of first leg of female, × 140; fig. 3, male genital organs, X 140; fig. 4, female, ventral view, × 100; fig. 5, heteromorphic third leg of male, X 140; fig. 6, section thru outer portion of foot of *Lampsilis luteola*, with encysted *Unionicola aculeata*, × 140.

Digitized by Google

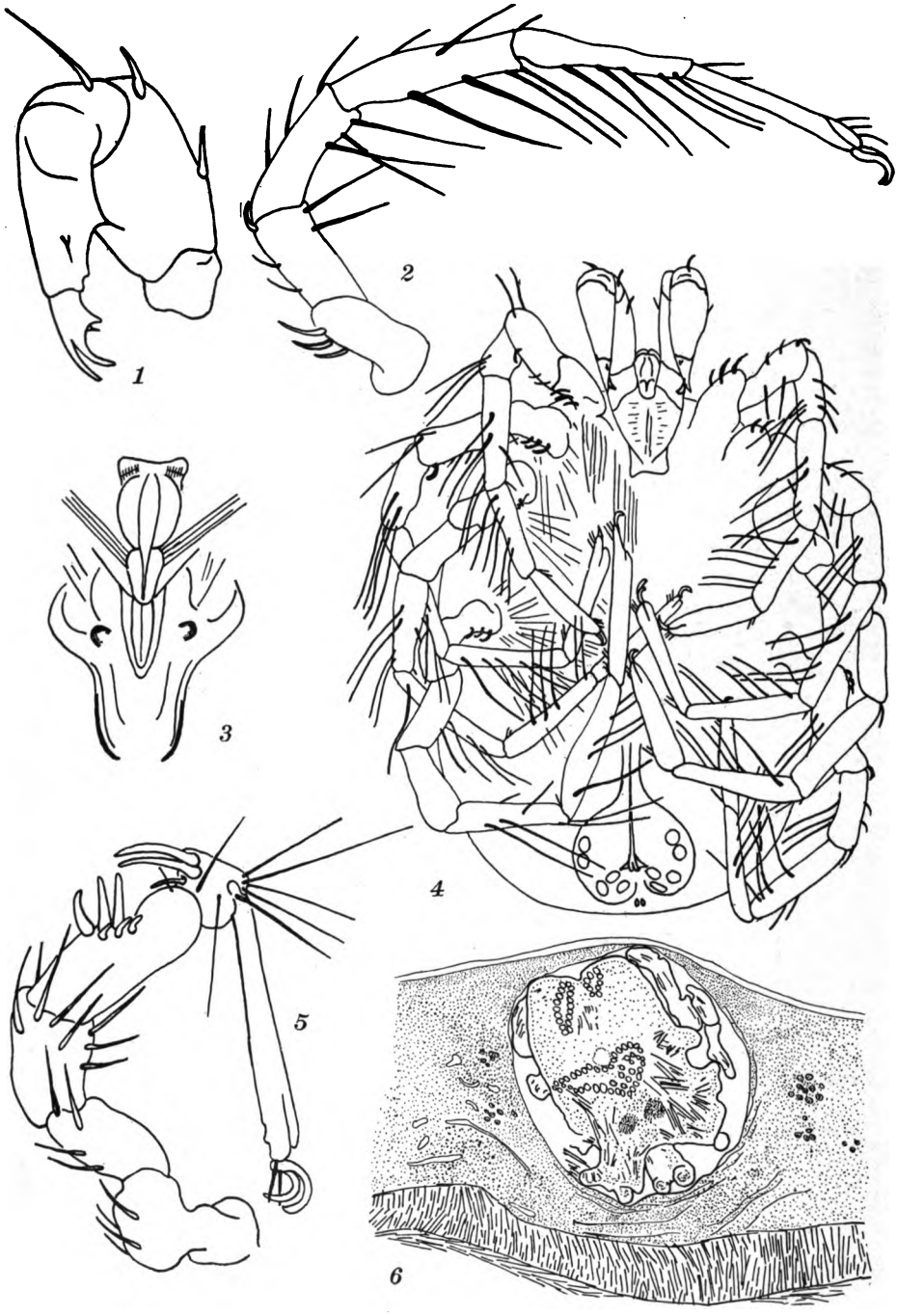


PLATE XII **FAUST**

Digitized by Google

Digitized by Google

DEPARTMENT OF NOTES AND REVIEWS

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In addition to these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

METHODS FOR STUDYING LIVING TREMATODES

Studies on the living animal are of great importance in morphological work on the Trematoda. Such studies have been entirely neglected by most workers on the group. I believe that an increased use of living material in the study of trematodes will make it possible to advance the knowledge of the group more rapidly and to avoid many errors. Material of living larvae and adult trematodes is easily obtained for class use, and its use adds greatly to the students' interest. Abundant material of sporocysts, rediae and cercariae can usually be obtained by the examination of freshwater snails and material for the study of adults from the intestines or lungs of frogs or snakes.

In miracidia or cercariae careful studies on locomotion reveal interesting specific characters, which may give hints of the type of host in which further development is carried on. The power of extention and contraction is so great in most trematodes, especially in larval stages, that a true conception of size and shape can be only gained from the living animal. The pattern of the excretory system can only be made out from living material. In fact it is almost impossible without careful studies in the living condition to define sufficiently the structure of a cercaria to insure specific determination. The amount of detail of structure which can be quickly obtained from the study of living trematodes is often surprising. In one small distome about 2 mm. in length it was possible from one living specimen not only to work out the connections of the reproductive ducts and to gain some idea, from the direction of the beat of the cilia, of the functioning of the parts, but also to make a camera lucida drawing under the oil immersion of the connections of the female ducts.

Agamodistomes and adults for live study should be transferred to slides in normal saline solution and covered with thin cover glasses.

Digitized by Google

The water should be slowly removed from the preparation with a piece of blotting paper until the pressure of the cover glass slightly flattens the fluke without injuring any of its structures. Such a preparation, when carefully sealed with vaseline, will often remain a whole day in perfect condition for study and can be examined even with an oil immersion lens. Sporocysts, rediae and cercariae are usually found in large numbers in the digestive gland of the gasteropod host. The fully developed cercariae should be mounted for study in the water from which the snails are obtained, and the sporocysts, rediae and immature cercariae in normal saline. By slowly removing the water from beneath the cover glass with blotting paper, cercariae so small that they are almost invisible to the naked eye can be slowed down and flattened so that they can be studied under the highest powers of the microscope. When dealing with small forms it is easier to make new mounts as the one being studied becomes too dry, than it is to try to make a preparation more permanent.

At every stage of compression different structures are brought out. Just before a cercaria goes to pieces in the process of drying the smaller tubules of the excretory system are distended and the movements of the flame-cells accentuated, so that they become clearly visible. By careful observations and the use of a number of preparations the number and position of the flame-cells and the relation of the tubules can be gradually traced until the whole pattern of the excretory system is understood and recorded. By this method I have been able to work out the excretory systems of miracidia, rediae, agamodistomes and small adult trematodes. The pattern of one system containing one hundred and twenty flame-cells was successfully solved. I know of no other way by which such a complicated excretory system could be worked out. The compound binocular microscopes recently put on the market have proven very helpful in the type of studies described above. This instrument is easy on the eyes and gives depth to the object observed. Several intra vitam stains have been tried. So far no method of intra vitam staining has been found which gives a better picture than the unstained animal.

WILLIAM WALTER CORT.

University of California.

Digitized by Google

A SUBSTITUTE FOR EUPARAL

In his original paper (La Cellule, 23, 427, 1906) Professor Gilson omitted the method of preparing this medium because of its difficulties and referred the matter to Grübler and Holborn. Under present circumstances it will perhaps not be a breach of etiquette to submit a method which yields a medium with similar index and properties. According to Gilson, Euparal is a solution of Sandarac in a mixture of Eucalyptol, Paraldehyde, and Camsal.

Paraldehyde and eucalyptol can be purchased of any chemist's supply house. They should be dry and neutral. If purchased from the druggist, who will probably substitute oil of eucalyptus, they should be redistilled, reserving the fraction, 119° to 124° for paraldehyde, and 175-177° for eucalyptol. The druggist's stock is in both cases about one-third something else.

Camsal is made by taking three parts of salol and two parts of camphor, and warming gently until completely liquid. Thereafter the mixture remains liquid, but should be kept well stoppered.

Sandarac as purchased is full of dust and ants. It may be purified as follows: 30 grams of sandarac are placed in a 200 cc. flask and 150 cc. of absolute alcohol added. Let stand with occasional shaking until dissolved. This mixture is rather sensitive to water vapor from the air and should be handled accordingly. Filter the solution thru a good filter paper into a 300 cc. flask. This is best done by resting the short funnel in the neck of the receiving flask and covering the whole with a bell jar under which some anhydrous calcium chloride is placed. Filtration is much more rapid than Mayer's albumin. The receiving flask is now fitted with a two-hole stopper. Thru one hole passes a glass tube in which a cotton filter has been placed; to catch dust, the rubber connections must of course be clean. This filter is connected to a calcium chloride drying tower to remove water vapor. The other hole is connected thru a receiving flask, if the alcohol is to be recovered, and thence to an aspirator. Air is passed while the solution of gum is warmed gently to 50-60°. Do not bubble air thru the solution, that being unnecessary and injurious. As the solution becomes thicker the temperature may be slowly raised to 70° and finally to 80° to remove the last of the alcohol. When the gum is moderately brittle on cooling the operation is ended.

To the gum in the same flask, add 20 cc. Eucalyptol, 10 cc. Paraldehyde, 10 cc. Camsal, cork and warm gently until a homogeneous solu-

Digitized by Google

tion is obtained. This gives a medium with an index n=1.483 to 1.486. The index can be raised or lowered slightly by varying the proportions used in making the solvent. Thus, the indices of the ingredients are about: Eucalyptol 1.456; Paraldehyde 1.39, this varies with the preparation used; Camsal 1.534; Sandarac 1.525. The essence d'euparal is, of course, the solvent mixture used above. The green tint mentioned by Gilson as due to a certain copper salt is probably copper abietinate which can be had of Merck or can be made of sufficient purity by any student in the organic chemistry laboratory.

In my experience there is less difficulty in preparing this medium than with some of the staining mixtures. It takes time but also little attention. Slides mounted several months ago are in excellent condition and as near as one can judge the medium acts like Euparal. Sections can be mounted from 80% alcohol, either with or without passing thru the essence.

E. S. SHEPHERD.

Geophysical Laboratory.
Washington, D. C.

CHROMOSOMES OF RANATRA SP?

During the summer of 1914 while working on the male germ cells of another type, I prepared and sectioned some testes from a species of *Ranatra* collected about Madison, Wis. The large number of chromosomes together with what seemed to be a very puzzling polymorphism of spermatocytes induced me to defer a further investigation till a later time. Recently the work upon this form has been resumed and has progressed to a point where a preliminary and tentative statement may be made.

The testes in the later nymph stages are especially valuable for sectioning as they present in many cases the whole history of the germ cells from the last spermatogonial divisions to mature spermatozoa. Sex organs from adults collected in the spring, and up to mid-summer are also generally favorable, but specimens taken in late summer and fall show very few division stages.

My first material was composed of several testes from animals collected in mid-summer and at that time believed to belong to but one species. All of these were prepared together for study. Observations

Digitized by Google

upon this mixed material show that there are at least two types of testes as regards the chromosomes.

The first type has spermatogonia with 40 chromosomes of various sizes; primary spermatocytes with 21, all of which divide equally; secondary spermatocytes with 21, two of which do not divide but pass directly into different spermatids each of which then possess 20 chromosomes. The two chromosomes which do not divide in the second division act as a typical XY pair and always appear near the center of the chromosome group in this division but their behavior is not sufficiently different to enable them to be identified before this stage.

The second type has spermatogonia provided apparently with 8 or 10 more chromosomes than the first type. The primary and secondary spermatocytes seem to have as a distinguishing mark a group of very small chromosomes near the center of the larger group. Neither the number nor the behavior of the chromosomes in the spermatids has been determined although some interesting conditions are suggested by the rather meager observations made to date.

Another interesting though not necessarily important fact is that among the individuals collected in July few possessed testes of the second type while a large percentage of those collected in September did possess cells of that type.

In addition to the interest attached to spermatogenesis these forms seem to offer an opportunity to determine possible correlation between the chromosome differences and somatic variations as soon as the individual origin of the two kinds of germ cells can be determined.

A. M. CHICKERING.

Beloit College.

NOTES ON COLLECTING AND MOUNTING ROTIFERS

C. F. Rousselet, the veteran English naturalist (J. Q. M. C., Nov. 1917) sums up methods which he has worked out for collecting, handling, preserving and mounting rotifers.

For a collecting stick he recommends a walking stick with a telescopic joint, with a ring net 6x5½ inches and 6 inches long, made of bolting silk No. 15 or 16. Silk lasts longer than mull and does not clog or shrink as it does.

Digitized by Google

The bottled materials collected are placed, on reaching home, in aquaria with 7x7 inch parallel sides one and one-fourth inch in depth from back to front. After a few hours the debris will have settled, and if a strong light be placed at one face of the aquarium the free-swimming rotifers will collect on the side toward the light, and can be discovered with a lens and be picked up with a pipette. In solid watch glasses these general collections can be examined quickly for new species with the low power of the binocular and unfamiliar forms transferred to the live-box or the micro-glass trough for special study.

Narcotising the mass of rotifers in the watch glass is readily effected by 1% cocaine. They may be killed and fixed by a drop of ½ to ⅛% osmic acid. They should be exposed to the osmic acid for a minute only and then removed to formalin of 2½% strength, changing it several times until well washed.

The sorting out of different species is done under the binocular by means of a bristle mounted in a suitable handle. They are then picked up with a fine pipette and placed in an appropriate micro-cell, and finally mounted in 2½% formalin.

The ringing of the micro-cells may be done as follows: first a thin ring of picture copal varnish; then several coats of Heath's cement (gold size-shellac-India rubber); finally finishing with three more coats of gold size.

METHODS OF PRESERVING CERTAIN MARINE BIOLOGICAL SPECIMENS

F. Martin Duncan (J. R. M. S., Dec. 1917) brings together methods which he has found most practical and successful in preserving marine plant and animal life and in preparing it for microscopic examination. Many of these methods are standard; but summarizing some of them may be of value.

Anaesthetising

Place the smaller and specially sensitive medusæ in just sufficient sea-water for free expansion and swimming, and add two drops of 1% solution of hydrochloride of cocaine, gently stirring with a glass rod. Repeat at five minute intervals until the tentacles do not contract when gently touched. Add 10-20 cc. of 4% formaldehyde solution, stirring for several minutes. Store in 10% formaldehyde. Do not allow specimens to remain in cocaine longer than absolutely necessary before adding the formaldehyde, as the former softens the jelly of the medusæ.

Digitized by Google

The author regards cocaine as, on the whole, the best anaesthetic for the most of the smaller forms of marine life. Solutions of coacine must be made anew since they do not keep well—becoming filled with fungoid growths.

Hydroid zoophytes, simple and compound ascidians, holothurians, anemones, and the like may be stupefied effectively with menthol. This is slow in action and does not simulate to contraction. The animals are submerged in clean sea-water and methol crystals are strewn over the surface. Their solution is slow, and in twelve or twenty-four hours depending on the size and sensitiveness of the animals and the amount of water, the specimens will be narcotised in an extended position, and may then be killed and fixed in any suitable fluid.

Fixing

The author prizes Bouin's fluid (Picric acid, saturated aqueous solution, 75 parts; formalin 25 parts; glacial acetic acid, 5 parts) as the best fixative for histological purposes. It has great power of penetration, kills quickly, and fixes well. It allows after treatment of the most varied sort. Next in desirability he considers saturated solution of corrosive sublimate.

Weak osmic acid (a few drops of a ½% solution added to the water in which the organisms are) is suggested for marine Protozoa. Radiolaria are effectively killed and fixed in corrosive sublimate. Sphærozoa give good results in equal parts of sea water and 70% alcohol with a trace of tincture of iodine added.

For Echinoderm larvæ an exposure of four minutes to a cold saturated solution of corrosive sublimate is recommended. For whole mounts dilute cochineal stain—as Mayer's alcoholic cochineal formula.

Small sponges are placed, on collection, in 1% solution of osmic acid and left there for five minutes, then transferred to strong alcohol and changed twice. Stain sections in Mayer's alcoholic cochineal.

Compound ascidians with contractile zooids may be handled to advantage by placing in clean sea water, narcotizing with methol, and then plunging for three to ten minutes in glacial acetic acid. Wash in 50% alcohol and pass thru successive grades to a preserving strength. Use no metal in the operation.

For small crustacea, both larvæ and adults, first treatment with 5% formaldehyde in sea water is recommended. Transfer to 70% alcohol.

Digitized by Google

For demonstration mounts it is necessary to guard against overstaining. Weak alcoholic picro-carmine cleared in turpineol is advocated as a means of staining.

THE SILVERMAN ILLUMINATOR FOR MICROSCOPES

This illuminator, invented by Professor Alexander Silverman of the School of Chemistry, University of Pittsburg, is a small, circular tube lamp which can be fitted quickly to any objective. It moves up and down with the barrel and furnishes a diffused and uniform illumination at the exact place where it is needed. It is suitable both for low and high power work, and may be used both for direct examination and for photography of opaque objects.

Much structural detail is revealed by this device which the older forms of illumination do not give. It is a low voltage tungsten lamp, and may be supplied either in colorless glass or in daylight (blue) glass. Its life is about 100 hours. There is no image of the source of illumination nor does the light strike the front of the lens except as reflected from the object.

The intensity of the light reaching the eye is lower than in other types of illumination, and yet because it is directed upon the spot observed the observer sees more. There is no glare, no waste light, no unduly contracted pupil, no unnecessary eye strain.

The lamps are manufactured by Ludwig Hommel & Co., Pittsburg, Pa.

Digitized by Google

Digitized by Google

Digitized by Google

CLASSIFIED ADS

For Frogs, Crawfish, Star-fish Perch, Clams, Turtles, Toads, etc., etc., send for latest price list.

THE ANGLERS CO.

913 W. Randolph St. Chicago, Ill.

Comstock Publishing Company

Ithaca, N. Y.

THE MICROSCOPE—12th Edition, 1917. Contains the old and also the new things in microscopy. S. H. Gage. Postpaid $3.00.

OPTIC PROJECTION—Especially full on the projection microscope and drawing. S. H. and H. P. Gage. Postpaid $3.00.

A Chemical Sign of Life

By Shiro Tashiro

A new method for determining whether a seed is alive or dead. $1.00, postage extra (weight 14 oz.)

One of "The University of Chicago Science Series"

The University of Chicago Press

CHICAGO 5857 Ellis Avenue ILLINOIS

Marine Biological Laboratory

Woods Hole, Massachusetts

BIOLOGICAL MATERIAL

1. ZOOLOGY. Preserved material of all types of animals for class work and for the museum.
2. EMBRYOLOGY. Stages of some invertebrates, fishes (including Acanthias, Amia and Lepidosteus), Amphibia, and some mammals.
3. BOTANY. Preserved material of Algae, Fungi, Liverworts, and Mosses.

Price lists furnished on application to
GEORGE M. GRAY, Curator
Woods Hole, Mass.

Correspondence solicited on special orders, e. g., living marine or aquatic material.

THE CAMBOSCO

1884-1918

A Biological House Manned by Biologists

SLIDES—Microscopical, by expert histologists, masters of the technique of sectioning, staining and mounting. The best men, in each branch, to be found in America or England. *No "Tyro" slides.* (Cat. B. 92.)

BOTANY—Preserved material, expertly collected. Lowest to highest Orders. (Cat. B. 92.)

ZOOLOGY—Preserved specimens, expertly collected. Class or Museum Material. (Cat. B. 92.)

GLASSWARE & PORCELAIN—The items you need, always in stock, the best for particular needs. (Cat. C. 94.)

HARDWARE—All lines, all styles and sizes, *Guaranteed.* (Cat. C. 94.)

It will cost you Three Cents and One Letter to ascertain our Current-Net-Itemized prices. To this extent, at least, may we serve you?

Cambridge Botanical Supply Company

Laboratory Equipment

WAVERLEY, MASSACHUSETTS

2 Lexington St. Established 1884

Ward's Natural Science Establishment

84-102 College Ave.

ROCHESTER, N. Y.

Material illustrating all branches of Natural Science.

Zoological Department. Material for exhibition or study; a large series of skeletons.

Anatomical Department. Models and special dissections.

Mineralogical Department. Minerals and rocks of all kinds. Collections and rare specimens.

Paleontological Department. Collections of fossils; choice specimens and casts.

Entomological Department. Insects of all orders, living pupae and supplies.

Microscope Slide Department. Over 2000 carefully prepared slides on all subjects.

Circulars from all Departments Free

Your correspondence is solicited

Members and Friends Will Find Our Advertisers Reliable

Digitized by Google

Powers & Powers

HIGH GRADE

Microscopic Slides

AND

Living Micro-Organisms

Living Amœba Proteus and Living Fresh Water Hydra.—We were the first in America to develop the rearing and shipping of these organisms to all parts of the continent. We ship to every State in the Union and even as far as Nova Scotia. Living delivery always guaranteed within the months of the school year. We have a private plant of our own especially adapted to these and other micro-organisms.

Microscopic Slides.—We have a growing list of high grade preparations for the microscope, chiefly in zoology. We endeavor to produce slides of unusual excellence. Many are quite unprocurable elsewhere.

Circulars and Prices Upon Application

POWERS & POWERS

Station A LINCOLN, NEBR.

LIVING AMOEBA

Zoology, Botany and Physiology

classes can be supplied with the living and preserved laboratory material required in the adopted textbooks. For scientific research and for museum exhibits, we make a specialty of supplying interesting species from Louisiana and the Gulf of Mexico.

All material guaranteed

Price lists and further information sent upon request.

SOUTHERN BIOLOGICAL SUPPLY COMPANY, Inc.

Natural History Building

NEW ORLEANS, LOUISIANA

THE CHICAGO BIOLOGICAL SUPPLY HOUSE

5505 Kimbark Ave.
CHICAGO, ILLINOIS

Biological Supplies of All Kinds

We have a free 1918 Calendar

Established 1883 Without Intermission 1916

A. A. SPHUNG

The Frog and Turtle Man

Live Frogs, Turtles, Crayfish, Clams, Earthworms. Also preserved materials shipped to all parts of the world.

Write for Price List
Lists Free

NORTH JUDSON, INDIANA

Digitized by Google

TO OUR PATRONS

We wish to thank you for your coöperation during the past year and for your patient consideration, as we have endeavored to supply the continued heavy demand for our instruments under unprecedented production conditions.

At this critical period in our nation's history we must each discover what our country requires of us; then do our particular bit, gladly, to help win the war as speedily as possible.

It has not been difficult for us to discover our bit, for optical instruments are the eyes of the modern army and navy, and we are bending all our energies to the endeavor to meet those requirements.

We are confident that you, too, are adjusting yourself to prevailing conditions, cheerfully and optimistically, for pessimism never promoted any cause. Yours is a vocation the country needs in wartime as well as in peace, and we hope to continue to assist you in the solution of your problems.

May we thus work together, then, for the best interests of humanity—first, to win the war; second, to maintain the momentum of all worthy activities in the nation, looking steadily to the day when the war shall cease.

Bausch & Lomb Optical Co.

NEW YORK WASHINGTON CHICAGO SAN FRANCISCO
LONDON ROCHESTER, N.Y. FRANKFORT

Leading American Makers of Microscopes, Photomicrographic and Projection Apparatus (Balopticons), Photographic and Ophthalmic Lenses, Binoculars and Other High Grade Optical Products.

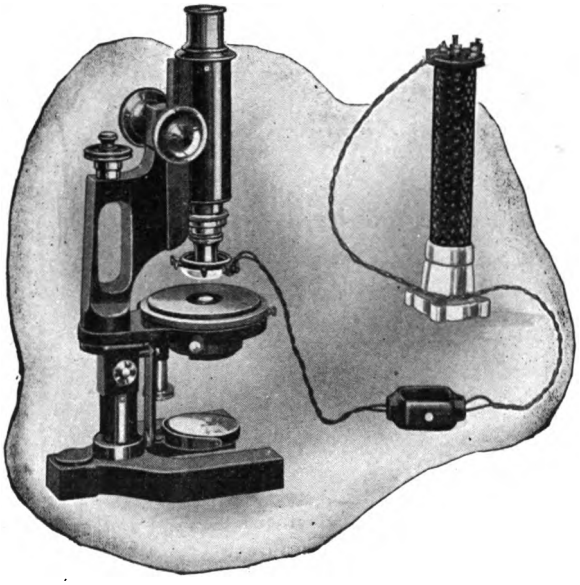
Members and Friends Should Patronize Our Advertisers

Digitized by Google

The

SILVERMAN ILLUMINATOR

Is indispensable for opaque work. It is the invention of Professor Alexander Silverman, School of Chemistry, University of Pittsburgh.



A small circular tube lamp surrounds the objective and furnishes a diffused and uniform illumination directly where it is needed. The Silverman Illuminator marks

A GREAT ADVANCE IN MICROSCOPE ILLUMINATION

Much structural detail which does not appear under the light of the present types of illuminators become visible through the use of the Silverman Illuminator.

The intensity of the light reaching the eye is lower than with other illuminators, yet the observer sees more. Hence the Silverman Illuminator *eliminates eye strain, eye fatigue and brain fag.*

The Silverman Illuminator *saves your time,* because it is placed on the microscope and connected, ready for service, in a few moments. No further adjustments after that. It moves up and down with the microscope tube.

Write for Bulletin 28-A

Ludwig, Hommel & Co.

947 Penn Ave. Pittsburgh, Pa.

Digitized by Google

WATSON
Microscopes and Lenses

MICROSCOPE STANDS. These in common with certain other of our productions are on "war service only," but

Before You Decide

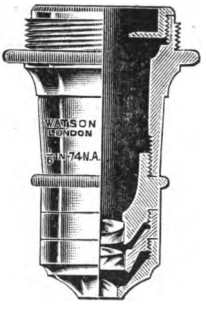
upon a new Microscope outfit, write for two little books that we issue. If you get these and study them, you will find the reason why all the leading British workers specify a

Watson Microscope With Watson Lenses.

OBJECTIVES. We are still open to supply certain of these. In this space, we have previously introduced to you the "Versalic" oil Immersion $\frac{1}{12}$ inch. *Now,* we want to draw your notice to another lens constructed from a similar computation,

A Really High Glass Objective at a Low Cost

(***You See It Here***)



We have been paid the greatest of all compliments with regard to this lens—that of extensive imitation.

Watson's 1-6 Inch Semi-Apochromat

Beautiful color correction, brilliant image, exceptionally long working distance. The latter is actually more than 1 m/m, so that an object can be focussed through an ordinary 3″ x 1″ slip.

N. A. O. 74 PRICE (during the war) $8.50

W. WATSON & SONS, Ltd.

(Established 1837)

Manufacturers to the British and Other Governments

313, High Holborn, London, W. C. I., England

Digitized by Google

RECREATIONS WITH THE MICROSCOPE

SPECIALIZATION is by no means a selfish isolation in a narrow line of interests, but rather it consists in making one's greatest interest the axis for the grouping of the others. The conditions of modern life have settled it for us that the only well-educated man is a specialist, one who knows something well, it matters not so much what, and has sympathy for other things.—*Dr. William F. Ganong.*

Every technical specialist in Microscopy has a sympathy for "other things"—amateur and elementary microscopy, also for all other departments of Nature Study.

You, Specialist, may please your "sympathy" and aid a Good Cause by active interest in

THE GUIDE TO NATURE

This monthly, illustrated magazine is devoted not to extreme knowledge but to "uncommon interest."

Subscription, $1.00 a year

THE AGASSIZ ASSOCIATION

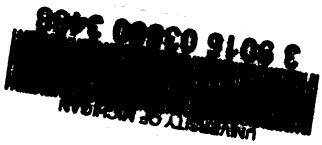
EDWARD F. BIGELOW, Managing Editor,
ArcAdiA,
SOUND BEACH, CONNECTICUT

Members and Friends Will Find Our Advertisers Reliable

Digitized by Google

Digitized by Google

Digitized by Google



GALLOWAY'S ZOOLOGY

3d Edition, Revised, Enlarged 255 Illustrations, 8 vo., 559 pp.

CLOTH $2.00 POSTPAID

By T. W. GALLOWAY, Ph. D., Professor of Biology,
Beloit College, Beloit, Wisconsin

"The book has many excellent characteristics."—School Science and Mathematics.

"The teacher may refer to it with confidence and cannot fail to do so with profit."—School Bulletin, N. Y.

"It is well balanced in its treatment of Zoology in its widest sense."—The Nation.

"One of the most complete and the best arranged treatises we have met with."—Journal of Education.

P. BLAKISTON'S SON & CO.

Publishers PHILADELPHIA

WE MANUFACTURE AND SELL:

High grade Microscopical and Dissecting Instruments
Museum and Laboratory Glassware.

WE IMPORT FREE OF DUTY AT LOWEST RATES:

Scientific Apparatus, Instruments and Preparations of every description.

WE PREPARE AND MOUNT:

Natural History Specimens, Biological Material.

Catalogues, Quotations and Information, on Application

OUR NEW BIOLOGICAL CATALOGUE AND SPECIAL STOCK LISTS ARE READY FOR DISTRIBUTION

THE KNY-SCHEERER CO.

Department of Natural Science

404 West 27th St., Cor. 9th Ave.

G. LAGAI, Ph.D. NEW YORK

Members and Friends Will Find Our Advertisers Reliable

Digitized by Google

INDEX TO ADVERTISEMENTS

The Anglers Co., Chicago I
Bausch and Lomb Optical Company, Rochester, N. Y. III
P. Blakiston's Son & Co. ..... Third P. Cover
Cambridge Botanical Supply Co., Waverley, Mass. I
The Chicago Biological Supply House, Chicago, Ill. II
Comstock Publishing Co., Ithaca, N. Y. I
Guide to Nature, Arcadia, Sound Beach, Conn. VI
The Kny-Scheerer Co., 404 West 27th St., New York ..... Third P. Cover
Ludwig Hommel & Co., Pittsburgh, Pa. IV
Marine Biological Laboratory, Wood's Hole, Mass. I
Powers & Powers, Lincoln, Neb. II
Southern Biological Supply Co., New Orleans II
A. A. Sphung, North Judson, Ind. II
University of Chicago Press I
Ward's Nat. History Establishment, Rochester, N. Y. I
W. Watson and Sons, 313 High Holborn, London, W. C. V

Members should bear in mind that our advertisers make possible a larger magazine than could otherwise be issued. We owe them our patronage as well as our thanks.

Nowhere will you find more reliable dealers in materials for the laboratory than those advertised in this magazine.

Digitized by Google